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<b>(54) Title:</b> PLANT GENE SPECIFYING ACETYL COENZYME A CARBOXYLASE AND TRANSFORMED PLANTS CONTAINING SAME  <b>(57) Abstract</b>  DNA sequences of an acetyl Coenzyme A carboxylase from plants are inserted into the genome of plants in sense or antisense orientation in order to inhibit expression of the gene product of the endogenous ACCase gene, resulting in reduced conversion of the enzyme's substrate, acetyl Coenzyme A, to fatty acid synthesis, leaving the substrate available for diversion into other biosynthesis pathways. One such diversion may be accomplished by providing the plant genome with genes specifying the synthesis of polyhydroxyalkanoate polymers.		

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PLANT GENE SPECIFYING ACETYL COENZYME A  
CARBOXYLASE AND TRANSFORMED PLANTS CONTAINING SAME

5 This invention relates to a plant gene specifying the enzyme acetyl Coenzyme A carboxylase (ACCase) and to plant genomes genetically transformed with the said gene. Particularly, but not exclusively, the invention relates to ACCase genes from plants of the Brassica species, especially Brassica napus (oilseed rape) and control of expression of the gene by Brassica plants which are genetically transformed with the gene or its antisense configuration.

10 Acetyl Coenzyme A carboxylase is one of the genes involved in the synthesis of oil by oil-producing crops such as oilseed rape. Variation of the expression of that gene leads to alteration in the quantity and/or quality of the oil produced.

15 An object of the invention is to provide a gene specifying ACCase in plants.

According to the present invention there are provided partial cDNAs specifying ACCase, isolated from seed of Brassica napus, having the nucleotide sequences set forth in Figures 6 and 12, and variations thereof permitted by the degeneracy of the genetic code.

20 The invention further provides the partial cDNA, isolated from wheat germ, having the nucleotide sequence set forth in Figure 4, and variants thereof permitted by the degeneracy of the genetic code.

25 Also provided by this invention is the full length genomic DNA specifying ACCase from Arabidopsis thaliana

having the nucleotide sequence set forth in Figure 8, and variants thereof permitted by the degeneracy of the genetic code.

5 The invention further provides the following clones, inserted in Escherichia coli, strain DH $\alpha$  hosts, which have been deposited with the National Collection of Industrial & Marine Bacteria, 23 St. Machar Road, Aberdeen, AB2 1RY, United Kingdom, on 25th March 1993, under the provisions of the Budapest Treaty on the  
10 Deposit of Microorganisms for Patent Purposes, details of which are as follows:

1. Plasmid pK111, Accession No. NCIB 40553
2. Plasmid pKLU81, Accession No. NCIB 40554
3. Plasmid pRS1, Accession No. NCIB 40555

15 The present invention also provides genetically transformed plants, plant cells and plant parts, containing a DNA of the invention or fragment thereof in sense orientation or a complete or partial sense or antisense variant thereof.

20 It is preferred that the plant be of a species which produces substantial quantities of oil, rather than starch. Such plant species are well known and are simply referred to as "oil-seed" crops and include, oilseed rape, canola, soya and sunflower. Methods for the  
25 genetic transformation of many oil crops are known; for example, transformation by Agrobacterium tumefaciens methods are suitable for most. Such methods are well-described in the literature and well-known and extensively practised in the art.

30 In our International Patent Application Number WO 92/19747, published on 12th November 1992, we describe the biosynthesis of polyhydroxybutyrate from the substrate, acetyl-CoA. This activity involves three enzyme- catalysed steps. The three enzymes involved are

$\beta$ -ketothiolase, NADP linked acetoacetyl-CoA reductase, and polyhydroxybutyrate synthase, the genes for which have been cloned from Alcaligenes eutrophus (Schubert et al, 1988, J Bacteriol, 170). In our international  
5 application we describe the cloning of these three gene into oil-synthesising plants.

However, the synthesis of fatty acids which are the building blocks of plant oils utilise the substrate acetyl Coenzyme A which is the same substrate required by  
10 the polyhydroxyalkanoate genes. By virtue of the present invention we provide means for down-regulating the fatty acid synthesis by inhibiting ACCase thereby leaving the acetyl CoA available for conversion to polyhydroxyalkanoates.

15 Methods for the regulation of gene expression are well-known in the art. Two principal methods are commonly employed, these being referred to loosely as "sense" and "antisense" regulation. In antisense regulation a gene construct is assembled which, when  
20 inserted into a plant cell, results in expression of a messenger RNA which is of complementary sequence to the messenger produced by a target gene. The theory is that the complementary RNA sequences form a duplex thereby inhibiting translation to protein. The complementary  
25 sequence may be equivalent in length to the whole sequence of the target gene but a fragment is usually sufficient and is more convenient to handle. In sense regulation a copy of the target gene is inserted into the plant genome. Again this may be a full length or partial  
30 sequence. A range of phenotypes is obtained from which individuals in which the expression of the protein encoded by the target gene is inhibited may be identified and isolated as may individuals where expression of the gene product is increased. Sense regulation using

partial sequences tends to favour inhibition. The mechanism is not well understood. Reference is made to European Patent Application No. 140,308 and United States Patent 5,107,065 which are both concerned with antisense regulation and International Patent Application No. WO 90/12084 which describes sense regulation. The invention permits the following genetic modifications to be effected:

1. The clones of the invention may be used to probe plant DNA ( genomic or cDNA libraries) to obtain homologous sequences. These may be truncated or full length cDNAs or genomic DNAs for ACCase genes from, for example, wheat, or oil crops such as rape, canola, soya, sunflower, maize, oil palm and coconut.

2. Partial cDNAs of rape seed ACCase may be used in conjunction with a plant-recognised promoter to create an expression cassette (partial sense or antisense) for use in transforming rape plants to down-regulate production of the ACCase enzyme. This will give plants with a lower oil content or oil of altered quality. The same cassette can be used to down-regulate the production of ACCase enzyme in other plants of the Brassica species. cDNAs isolated from other crops can be used to create expression cassettes (partial, sense or antisense) for use in transformation of these crops in order to modify the oil content.

Down-regulation of oil synthesis (in rape or other oil crops) can be used to divert the substrate, acetyl Coenzyme A, into synthesis of alternative storage materials such as starch, protein, or novel polymers introduced by genetic modification, for example polyhydroxyalkanoates.

3. Full length clones of rape or Arabidopsis ACCase DNA can be used to create expression cassettes, either

with powerful promoters, or by inserting extra gene copies, to promote over-expression of ACCase in rape or other oil crops, leading to plants with enhanced oil content in the seed. The ACCase DNA may also be put under the control of a seed-specific promoter such as the napin promoter, which has a different window of expression from the ACCase promoter during seed development. In this way the period over which ACCase is expressed in the developing seed is extended, and the oil content of the seeds increased.

4. Genomic DNAs of rape ACCase can be used to recover the promoter of the ACCase gene. This promoter can be used to generate RNA in a tissue-specific and developmentally regulated fashion. The promoter so generated may promote the expression of ACCase, or it may control the expression of a gene construct placed after it (for example the structural gene of a different enzyme) which will then be expressed specifically in the developing seed.

5. The full length cDNA and genomic DNA of rape or Arabidopsis ACCase contain a sequence between the translation start site and the N-terminal sequence of the mature protein, known as a "transit peptide" sequence. This directs the gene product to the plastids and is cleaved off during import of the protein into the plastids. This transit peptide sequence may be used in gene fusions to direct different gene products to the plastids.

6. Monocotyledonous plants, such as wheat, barley, maize and rice, are normally sensitive to the aryloxyphenoxy- propionate and alkylketone herbicides to which the dicotyledonous plants are normally resistant. Monocots with resistance to these herbicides may be created by:

(a) transforming ACCase from a dicotyledonous species such as rape and Arabidopsis, into the monocot genome;  
(b) overexpression of the ACCase in a monocot; or,  
(c) mutagenesis of ACCase and insertion of the mutant gene into a monocot.

7. It is believed that ACCase activity exists in both the plastid and the cytosol. Partial cDNAs of rape seed ACCase of this invention may be used in conjunction with a plant-recognised promoter to create an expression cassette (partial sense or antisense) for use in transforming plants to down-regulate production of the cytosolic ACCase. This will alter oil quality by inhibiting production of long chain fatty acids) chain length greater than about C18).

8. A second plastid form of ACCase has been identified in plants. This ACCase is composed of dissociable sub-units for transcarboxylase, biotin carrier protein (BCP) and biotin carboxylase (BC). The transcarboxylase gene is encoded by the chloroplast genome; BCP and BC are nuclear encoded. Sequence homology between the cDNAs of the invention and the BCP and BC may be used to isolate BCP and BC. Sense and antisense constructs may be raised against BCP and BC in order to effect down-regulation of these genes.

9. The cDNAs of the invention may themselves have sufficient homology with the BCP and BC genes to be used directly for the down-regulation of these genes.

We have prepared a poly dT primed cDNA library from developing rape seed and have obtained another from developing wheat embryo. These libraries have been probed with DNA fragments isolated earlier from a partial length maize leaf ACCase DNA (pA3) and partial length cDNA clones specifying rape seed ACCase (pRS1) and wheat germ ACCase (pK111) have thereby been selected and sequenced.



A DNA fragment isolated from the partial length rape ACCase DNA was then used to probe a genomic DNA library prepared from Arabidopsis thaliana and a full length Arabidopsis genomic DNA selected and sequenced.

5       The sequence of the Arabidopsis genomic DNA was used to generate specific probes by PCR. These were used to screen a random primed cDNA library from rape seed and two further rape ACCase partial cDNAs were thus isolated.

10       The full length Arabidopsis ACCase genomic DNA may then be used to probe a genomic library from rape and the full length rape ACCase genomic DNA selected and sequenced.

That the clones were indeed of ACCase genes was confirmed as follows:

15       The deduced amino acid sequence for wheat ACCase cDNA shows complete homology in four regions of sequence to the amino acid sequences obtained from four peptides isolated from the ACCase enzyme purified from wheat embryo. The deduced amino acid sequence shows high homology with both  
20       the rat and chicken ACCase genes. High homology at the amino acid level with maize leaf ACCase was found, with two sections of 48 amino acids completely conserved.

25       The deduced amino acid sequence from the rape seed partial cDNA (pRS1) sequence shows high homology to the sequences of the maize leaf cDNA and the chicken, rat, yeast and algal ACCase genes.

30       The deduced amino acid sequence from the Arabidopsis genomic DNA shows high homology with the rat, chicken and yeats ACCase genes. High homology with the amino acid sequence of the rape seed ACCase partial cDNA (pRS1) was found, with one section of 48 amino acids almost completely conserved.

The invention will now be described with reference to the accompanying drawings which show:

Figure 1 shows the elution profiles of wheat embryo ACCase from Q-Sepharose (Figure 1A) and Blue-Sepharose (Figure 1B) during purification of the enzyme. The dotted line represents the sodium chloride gradient concentration and the activity of ACCase, represented by the boxes, was measured as described hereinbelow;

Figure 2A shows an SDS PAGE gel of wheat embryo ACCases showing the alteration in mobility caused by the binding of streptavidin. Lane 1 contains 500ng myosin (200kDa); lane 2 contains 10 $\mu$ l Post Blue-sepharose material without Streptavidin; and, lane 3 contains 10  $\mu$ l Post Blue-sepharose material with streptavidin. ACCase is indicated by asterisks (\*) at its normal migration and that of the ACCase/streptavidin complex respectively.

Figure 2B shows an SDS PAGE gel of purified wheat embryo ACCase, with the 220kd band taken for sequencing indicated. Lane 1 contains 1 $\mu$ l Post Blue-sepharose material and lane 2 contains 10  $\mu$ l Post Blue-sepharose material;

Figure 3 shows a comparison of four sections of amino acid sequence deduced from the pK111 wheat ACCase cDNA with the amino acid sequences obtained from four peptides isolated from the purified wheat embryo ACCase enzyme;

Figure 4 shows the sequence of the sense strand of the wheat embryo ACCase clone pK111, with three-phase translation shown. The sequences homologous with the peptide amino acid sequences are underlined;

Figure 5 shows a dot matrix plot of the deduced amino acid sequence of wheat ACCase clone pK111 against that of the maize ACCase clone pA3;

Figure 6a shows the derived amino acid sequence from the rape cDNA encoding the transcarboxylase domain of ACCase. The amino acid sequence is translated from the first open reading frame shown pictorially. The full

vertical lines represent stop codons and the half vertical lines ATG sequences. Figure 6b shows the nucleotide sequence of the cDNA clone pRS1, corresponding to the trans carboxylan domain of ACCase.

Figure 7 shows the rape transcarboxylase domain comparison with known ACCase sequences. The Dot Matrix (DNA Strider, Stringency 9 Window 21) of derived rape ACCase amino acid sequence (transcarboxylase domain) is compared against rat, yeast and algal (Chlorella) ACCase.

Figure 8 shows the 5' sequence from the sense strand of the Arabidopsis genomic subclone pKLU81, with three phase translation shown.

Figure 9 shows the 3' sequence from the sense strand of the Arabidopsis genomic subclone pKLU81, with three-phase translation shown.

Figure 10 shows a comparison of the Arabidopsis pKLU81 5' translated open reading frame with the sequences of rat and chicken ACCase genes obtained from SWISSPROT database.

Figure 11 shows the assignment of domain order to higher plant ACCase. Figure 11A is a schematic diagram showing the yeast ACCase domain orders relative to the sequenced regions (hatched boxes) of the Arabidopsis genomic clone. The areas of sequenced genomic clone are named A-F for easy identification in the text.

In figure 11Bi) the translated open reading frame from area Aii is shown in direct comparison with a region from the biotin carboxylase domain of yeast. Boxed regions represent amino acid identity.

In figure 11Bii) the translated open reading frame corresponding to the biotin binding site in area C is shown in direct comparison with the biotin binding site of yeast. Boxed regions represent amino acid identity.

Figure 11Biii) shows a DNA sequence comparison by dot matrix (DNA Strider, Stringency 15 Window 23) of the rape transcarboxylase domain of ACCase and areas E/F from the

Arabidopsis genomic clone.

Figure 11C shows the nucleotide sequences of Regions A, Aii, B, C, D, E, F, Arabidopsis genomic clone pKLS2.

Figure 12 shows the rape ACCase biotin binding domain sequence.

5 Figure 12Ai) shows the derived amino acid sequence from the rape cDNA encoding the ACCase biotin binding domain. The actual biotin binding site is shown underlined.

10 Figure 12Aii) shows the direct comparison of the biotin binding site with that of the corresponding sequence of yeast ACCase. The boxed regions represent amino acid sequence identity.

15 Figure 12B shows the dot matrix comparison (DNA Strider, Stringency 9 Window 21) of derived rape ACCase amino acid sequence (biotin binding domain) against yeast ACCase.

Figure 12C shows the full combined nucleotide sequence of pRS6 and pRS8.

20 Figure 13 shows ACCase Southern blot analyses of rape and Arabidopsis genomic DNA. Restriction endonuclease digested DNA was hybridised to the Arabidopsis ACCase genomic clone by Southern blot. Hybridisation and washing conditions were carried out as described in materials and methods. The blot shown was exposed for 5 days, further exposure provided no extra information. Both  $\lambda$  HindIII and OX 174 HaeIII DNA markers (indicated on the left hand side) were run on the same 1% gel and viewed by ethidium bromide staining/UV.

25 Figure 14 shows Northern blot analysis of rape ACCase. In Figure 14A the graph shows the oil content as total fatty acid (mg/seed) in relation to the stage of rape embryogenesis. Details of the analysis method are presented in materials and methods. The three Northern blots shown, relating to the different stages of embryogenesis, are all derived from the same blot after

30

successive stripping. The probes used were as indicated in the text and the amount of polyA+ RNA was 5µg for each stage. Hybridisation and washing conditions were as in materials and methods. Exposure was for 7 days.

5 In Figure 14B the probe used in the Northern blot shown was the rape transcarboxylase domain cDNA derived from an embryo library. 1µg of poly A+RNA from 29 days post anthesis embryos and young leaf was used for the blot. Hybridisation and washing conditions were as in  
10 materials and methods. Exposure was for 7 days. Molecular weight markers were viewed by ethidium bromide/UV.

#### MATERIALS AND METHODS

##### 1.0 Protein purification and amino acid sequence data.

##### 1.1 Assay for ACCase

15 Acetyl CoA Carboxylase activity was assayed by incorporation of radioactivity from <sup>14</sup>C- bicarbonate into non-volatile malonyl CoA (Hellyer et al 1986).

##### 1.2 SDS Poly-Acrylamide Gel Electrophoresis

20 All SDS PAGE gels consisted of a 3% stacking gel with a 7.5% running gel on a mini Biorad Protean gel kit unless otherwise stated. The buffer system used was that of Laemmli et al (1970) unless otherwise stated. All gels used in separating peptides for sequencing were pre-run in the presence of 200µM thioglycolic acid in the running  
25 buffer.

##### 2.0 Cloning for Wheat/Rape/Arabidopsis ACCase

##### 2.1 Preparation of competent XL1-Blue and KW251

Escherichia coli cells XL1-Blue and KW251 cells were grown overnight in 50 ml LB media/0.2% Maltose/50µg/ml  
30 Tetracycline/10 mM MgSO<sub>4</sub>. The cells were spun down at 3000 g for 10 mins and the cell pellet taken up in 2.5 ml 10 mM MgSO<sub>4</sub> and stored at 4°C. Cells were used fresh for primary screening and no older than one week for subsequent screening.

##### 2.2 cDNA Libraries

##### 2.2.1 Wheat

The cDNA library used (gift of Dr Charles Ainsworth, Wye College, London) was generated using the pooled RNA from whole developing grain of Chinese Spring harvested at 3,5,7,10,15,25,30 and 35 days post anthesis. The cDNA was cloned into the EcoRI/XhoI site of  $\lambda$ -ZAP II (Stratagene) and the host bacteria used was XL-1Blue (see 2.1 for preparations of cells).

### 2.2.2 Rape

#### (i) cDNA Library from polyA+ RNA

The cDNA library used was generated using the mRNA isolated according to the method of Logemann et al (1987) from mid stage developing Jet neuf rape embryos (harvested at approximately 35 days post anthesis). The 1st strand synthesis was carried out using poly dT primers according to the manufacturers instructions (Amersham International). The resulting cDNA generated was cloned into the EcoRI/XhoI site of  $\lambda$ -ZAPII as recommended by the manufacturers (Stratagene). The host bacteria used was XL-1Blue (see 2.1 for preparation of cells).

#### ii) Random primed library

5  $\mu$ g of poly A+ mRNA from 35 day old (Post anthesis) Jet neuf rape embryo was used for the construction of a random primed cDNA library. The double stranded cDNA was prepared using a 1 in 10 dilution of pd(N)6 primers (0.74 $\mu$ g/ $\mu$ l) according to the instructions provided with Time Saver<sup>TM</sup> cDNA synthesis kit (Pharmacia). The library was prepared in  $\lambda$ ZapII and packaged with Gigapack II Gold packaging extract (Stratagene). The host E.coli strain used was XL-1 Blue (Stratagene).

### 2.3 Genomic Libraries

The Arabidopsis thaliana library used (a gift from Dr John Cowl, John Innes Institute, Norwich) was derived from leaf total DNA in  $\lambda$  FIX II and the host bacteria used was E.coli KW251 (see 2.1 for preparation of cells).

#### 2.4 Probe preparation and labelling

Plasmid DNA from pA3/DH5 $\alpha$  (ICI derived) and pRS1/DH5 $\alpha$  (see results for a description of pRS1) was prepared by the Quagen tip method. Probe for the screening of Wheat and Rape cDNA libraries was generated by the digestion of 10 $\mu$ g pA3 with 20 U EcoRI or Hind III (New England Biolabs). The fragment isolated from the probe was 2.7 and 1.54 kb in length respectively. Probe for the screening of the Arabidopsis genomic library was generated by a Xho I/Pst I (10 U of each) double digest of 10 $\mu$ g pRS1 to give an isolated fragment size of 1.2 kb. All digests were carried out in Pharmacia's "one-Phor-All Buffer PLUS" at 37°C for 3 hours. Digests were separated by 1% TAE buffered agarose gel electrophoresis and the required fragments cut out from the gel. The DNA was obtained from the gel slice using the method recommended by GeneClean II (Bio 101). DNA concentration was determined by spectrophotometry.

The probes (200-300 ng) were radio-labelled with p<sup>32</sup>dCTP using the Megaprime kit as recommended by the manufacturers (Amersham International) to a level of 5 x 10<sup>9</sup> dpm/ $\mu$ g. Un-incorporated label was removed using Biospin chromatography columns (Biorad).

Just before use for hybridisation the radio-labelled probe was boiled for 5 minutes and placed on iced water for 2 minutes before being added to hybridisation buffer at 65°C.

#### 2.5 cDNA library primary screening

For the Wheat cDNA library 300,000 pfu's and the rape random primed and poly dT primed cDNA library 150,000 pfu's were added to 2 ml of competent XL1-Blue cells (150,000 pfu's/2 ml) mixed and incubated at 37°C for 20 minutes. The culture was then added to 30 ml top agarose (150,000 pfu's/ 30 ml) which had been melted and held at

50°C, mixed briefly and poured onto pre-warmed (37°C) large LB plates (243 x 243 x 18 mm). Plates were left at room temperature for 10 minutes and incubated overnight at 37°C. The plates were finally incubated at 4°C for 30 minutes.

Square sheets of nitrocellulose were carefully placed onto the surface of each plate and allowed to soak in for 30 seconds, peeled off and placed onto 3 mm blotting paper soaked in denaturing buffer (1.5 M NaCl, 0.5 M NaOH) for 2 minutes. To neutralise the filters each was subsequently placed for 5 minutes onto 3 mm paper soaked in neutralising buffer (1.5 M NaCl, 0.5 M Tris pH 7.4) and finally for 5 minutes on 3 mm paper soaked in X2 SSC. A second lift of 2 minutes was also carried out and treated in the same way. To immobilise the blotted DNA each filter was placed in a vacuum oven for 30 minutes.

The filters were incubated in pre-hybridisation buffer (50 mls X6 SSC, X1 Dendhart's, 0.5% SDS, 0.05% sodium pyrophosphate, 50 µg/m<sup>1</sup> herring sperm DNA with constant mixing for 3 hours at 65°C at which point the buffer was discarded. The radio-labelled probe (see 2.4) was added to 10 ml hybridisation buffer (50 mls X6 SSC, X1 Dendhart's, 0.5% SDS, 0.05% Sodium Pyrophosphate, 1mM EDTA) previously equilibrated to 65°C. The filters were incubated with constant mixing for 14 hours at 65°C and the hybridisation buffer/probe removed but retained at -20°C for the subsequent screens.

To wash off the un-bound probe the filters were washed 4 times with X1 SSC, 0.1% SDS for 30 minutes at 65°C. Filters were air dried and exposed to film overnight. Positive plaques were located and pulled out from the plate using the wide end of a 1 ml gilson tip. Only plaques that showed up positive on both lifts (30 seconds and 2 minute lifts) were used. The plug was placed into



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500  $\mu$ l SM buffer with 10 $\mu$ l chloroform and incubated at room temperature for 2 hours with occasional mixing. The suspension was spun for 5 minutes on a bench top centrifuge and the supernatant containing the pfu's retained.

#### 2.6 Genomic library primary Screening

The methods used were as already described (see 2.5) but in a genetic manipulation isolation unit  $2 \times 10^4$  were screened in total on 2 plates.

#### 2.7 cDNA and Genomic Secondary Screening

50-200 pfu's in 200 $\mu$ l SM buffer were added to 200 $\mu$ l of competent XL1-Blue cells mixed and incubated at 37°C for 20 minutes. The culture was then added to 3 ml melted top agarose at 50°C, mixed briefly and poured onto pre-warmed 37°C) small LB plates (850 mm diameter). Plates were held at room temperature for 10 minutes and incubated overnight at 37°C. The plates were finally incubated at 4°C for 30 minutes.

Pre-hybridisation and hybridisation was carried out in the same way as that in the primary screen (see 2.5), using the same probe/hybridisation buffer boiled for 5 minutes before use.

The procedure for lifting, preparing, probing, washing and exposing the nitro-cellulose filters was essentially the same as that already described (see 2.5).

The positive plaques were removed as a plug using the wide end of a 200 $\mu$ l Gilson tip, placed into 500 $\mu$ l SM buffer with 10 $\mu$ l chloroform and incubated at room temperature for 2 hours with occasional mixing. The suspension was spun for 5 minutes on a bench top centrifuge and the supernatant containing the pfu's retained.

#### 2.8 cDNA and Genomic tertiary screening

The method was essentially the same as that for the

secondary screen (see 2.7) using only 10-20 pfu's per plant. Exposure of the nitrocellulose filters was only required for 2 hours in this instance.

#### 2.9 Isolation of DNA from positive plaques

5        Plasmid rescue for cDNA clones was carried out as described by the Stratagene protocol for "in vivo excision of pSK from  $\lambda$ -ZAPII clones". The DNA from the pSK derived clones was prepared in large quantities using the Quagen tip method.

#### 10        2.10 Preparation of Genomic DNA from positive plaques

One positive plaque was removed from a plate of the positive pfu's from the tertiary screen and incubated with 500  $\mu$ l fresh KW 251 cells (see 2.1 for method of cell preparation) at 37°C for 20 minutes. Pre-warmed LB media (50 ml at 37°C) was added in addition to 500  $\mu$ l 1 M  $MgSO_4$  and incubated with mild shaking at 37°C for 5-7 hours. Following the 5-7 hours, 250  $\mu$ l Chloroform was added to the culture and incubated for a further 15 minutes at 37°C. Cell debris was spun out at 10,000 g and DNase/RNase added to the supernatant to a final concentration of 1  $\mu$ g ml<sup>-1</sup> and further incubation at 37°C for 30 minutes. 5 g Polyethylene Glycol 8000/3.2 g NaCl was added slowly to the supernatant at 4°C overnight with constant stirring.

25        The resultant suspension was pelleted at 10,000 g (4°C) and taken up in 5 ml 20 mM Tris-HCl pH 7.4/100 mM NaCl/10 mM  $MgSO_4$ . The solution was then subjected to 3-5 chloroform extraction's and 3-5 1:1 Phenol:Chloroform extraction. To precipitate the DNA an equal volume of isopropanol (-20°C) was added and left on ice for 30 minutes. The precipitated DNA was pelleted at 10,000 g and washed in 70% Ethanol (-20°C) before being pelleted again. The DNA was resuspended in 300  $\mu$ l T<sub>10</sub>E<sub>1</sub> buffer.

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Subcloning was carried out according to the method used by Sambrook et al (1989).

### 2.11 Sequencing of DNA clones

Sequencing was carried out by the manufacturer's recommended methods for the machine used (Applied Biosystems Inc 373A DNA sequencer). Both forward and reverse primers (-21 m13 and M13RP1) were used initially for all clones. Oligonucleotides (20 mers) were generated and used to further sequence pRS1 (rape ACCase clone). pK111 (Wheat ACCase clone) was subjected to nested deletions by the recommended method (Pharmacia, "d.s. Nested Deletion Kit") and sequenced by a combination of forward and reverse primers and generated oligonucleotide priming. Computer analysis of DNA sequence was carried out using the SEQNET package from the SERC facility at Daresbury and DNA Strider.

### 3. Northern blot analysis

Poly A+ mRNA was prepared from either 5g young leaf or 5g embryos harvested at 15, 22, 29, 36, 42 and 49 days post anthesis using the recommended procedure (Pharmacia mRNA purification kit). 1-5 ug was loaded on to a 1% formamide/formaldehyde agarose gel for electrophoresis. The Northern blot procedure was as described previously (Elborough et al 1994).

### 4. Southern blot analysis

Total DNA isolated from rape and Arabidopsis leaves (10ug and 2ug/digestion respectively) was digested with EcoR1, HindIII and BamHI separately for 8 Hrs. The DNA was separated by TAE agarose electrophoresis, blotted and hybridised to radiolabelled probe as described by Sambrook et al.

## RESULTS

### 1.1 Partial purification of ACCase from Wheat germ

Partial purification of Wheat ACCase was carried out

- 18 -

essentially using the method previously described by Egin-Buhler et al (1980) with several modifications.

All operations were carried out at 4°C unless otherwise stated. All buffers used contained 14 mM  $\beta$ -mercaptoethanol and 0.3 mM EDTA.

5        6 x 25 g of dry Avalon Wheat germ was ground in a coffee grinder for 15 secs. 200 ml 100 mM Tris-HCl pH 7.5 was added to each and polytroned on full speed for 1 minute. The homogenate was stirred for 15 minutes and  
10       spun at 20,000g. The supernatant was stirred with 25g wet weight Dowex 50 previously equilibrated with 100 mM Tris-HCl pH 7.5 for 15 minutes. The suspension was filtered through cheese cloth and 10% Polyethyleneimine at pH 7.5 added to 0.03% w/v dropwise whilst stirring. After  
15       15 minutes the suspension was spun again at 20,000 g. Powdered  $(\text{NH}_4)_2\text{SO}_4$  was added to a final saturation of 60% and stirred for 1 hour. After spinning at 20,000g the pellets were resuspended in 100 ml 100 mM Tris-HCl pH 7.5/100m M NaCl. The supernatant was dialysed for 1 hour  
20       against 5 litres 100 mM Tris-HCl pH 7.5/100 mM NaCl and subsequently overnight with fresh buffer (5 litres). Powdered  $(\text{NH}_4)_2\text{SO}_4$  was added to a final saturation of 25% and stirred for 1 hour spun at 20,000 g and the supernatant brought up to 70% saturation. After  
25       centrifugation the resulting pellet was resuspended in 50 ml 20 mM Tris-HCl pH 7.5, 20 mM NaCl and dialysed with 3 x 1 hour changes against 5 litres 20 mM Tris-HCl pH 7.5, 20 mM NaCl/20% glycerol. The resultant suspension was diluted to a conductivity of  $<4.3 \times 10^{-3} \text{ cm}^{-1}$  and stirred  
30       slowly with 150 ml of pre-equilibrated Q-sepharose (in 20 mM Tris-HCl pH 7.5 20 mM NaCl/20% glycerol) for 2 hours. The unbound protein was removed using a sintered glass funnel and the matrix washed with 10 volumes of 20 mM Tris-HCl pH 7.5, 20 mM NaCl/20% glycerol. The slurry was

packed into a 10 cm diameter Pharmacia column. Protein was eluted from the column using a gradient of 60-500 mM NaCl/20 mM Tris-HCl pH 7.5/20% glycerol (see figure 1A for elution profile) at 100 ml hr<sup>-1</sup> collecting approx 9 ml fractions. Every other fraction was assayed for ACCase activity, the most active fractions pooled and brought to 50% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> saturation. The pellet after centrifugation was taken up in a minimal volume (approx 100 ml) of 20 mM Tris-HCl pH 7.5, 5 mM MgCl, 20% glycerol to give >4.6 x 10<sup>-3</sup> cm<sup>-1</sup> conductivity. This was incubated with 100 ml pre-equilibrated Blue-sepharose (in 20 mM Tris-HCl, pH 7.5/5 mM MgCl/ 20% glycerol) with mixing for 2 hours. The matrix was washed with 10 volumes of 20 mM Tris-HCl pH 7.5/5 mM MgCl/20% glycerol using a sintered glass funnel. The washed matrix was packed into a 10 cm diameter Pharmacia column and the protein eluted from the column with a 60-500 mM NaCl/20 mM Tris-HCl pH 7.5/5 mM MgCl/20% glycerol gradient (see Figure 1 B for elution profile) at 100 ml 1 hour taking 9 ml fractions. The pooled active fractions (post-Blue-sepharose material) were stored frozen at -70°C.

#### 1.2 Identification of approx. 220 kDa protein as biotin containing

The dominant 220 kDa a band in the post Blue-sepharose material was identified as ACCase by both its ability to change mobility during SDS PAGE in the presence of streptavidin and its estimated molecular weight (Egin-Buhler et al. (1980). SDS PAGE X5 loading buffer (5μl) was added to 20ul post Blue-sepharose material, boiled at 100°C for 2 mins. and 1μl of a 5mM Steptavidin stock added immediately. The solution was incubated at 650°C for 5 mins. and loaded onto an SDS PAGE gel next to myosin (Mr 200kDa) and untreated post Blue-sepharose material sample for comparison (see Fig 2A). Streptavidin

clearly reduced the mobility of the 220kDa band, indicating that it is biotin containing. The only known biotin enzyme with a MW of 220kDa is ACCase.

### 1.3 Generation and sequencing of wheat ACCase peptides

5           A sample of post-Blue sepharose material estimated to contain approx. 400pM (80 $\mu$ g) of ACCase, as determined by comparison with known concentration standards, was loaded onto an SDS PAGE prep gel (see 1.3 for method and Fig 2B for appearance of sample). The running buffer was fresh  
10           and had a reduced level of SDS (0.035% SDS). Chromaphor green (Promega) was added at 1:1000 dilution to the upper tank during electrophoresis to allow the visualisation of protein. the ACCase protein band at approx. 220 kDa was cut out of the gel, frozen and stored at -20°C overnight.  
15           The gel slices were trimmed of excess acrylamide and loaded on to one well of a 3mm thick large Biorad Protean gel. The gel slices once loaded were overlaid with Endoproteinase LysC (Promega) at 6.5% protein concentration in 50% glycerol/0.125M Tris pH 6.8,/0.1%.  
20           SDS/3% B-mercaptoethanol/0.005% Bromophenol Blue. The gel was run until the protein was at the stacker interface at which point electrophoresis was stopped for 1hr at room temperature. Electrophoresis was resumed until the dye front reached the bottom of the gel. Peptides were  
25           semi-dry blotted into ProBlot (Applied Biosystems Inc.) according to manufacturers instructions. Rapid Coomassie staining of the blot (according to ProBlot instructions) identified peptide fragments which were excised from the membrane and loaded onto an ABI 477A pulse liquid protein  
30           sequencer. Sequence data was obtained at an amino acid level of 10-20pM (see Fig 3).

Sequence data was obtained for 4 peptides, yielding stretches of N-terminal amino acid sequence of 17, 18, 9 and 20 amino acids (Fig 3).

## 2. ACCase clone isolation and sequencing

### 2.1 Wheat ACCase cDNA

5 A wheat cDNA library was probed with a 2.7 kb EcoR1 fragment, and a 1.54 kb HindIII fragment of the maize partial cDNA clone pA3 which contains 4.5 kb of the 3' maize ACCase. This yielded a 1.85 kb clone inserted between the Eco R1 and XhoI site in the multi cloning cassette of pSK. The DNA was recovered by plasmid rescue in the host strain DH5 $\alpha$ . This clone was denoted pK111.

10 The nucleotide sequence data of this partial cDNA, with the derived amino acid sequence from the three reading frames is shown in Figure 4.

15 Figure 4 also shows that sections of pK111 show complete homology with the amino acid sequence of the 4 peptides isolated from the purified wheat germ enzyme, providing good evidence that the cDNA does indeed code for wheat embryo ACCase.

20 A dot matrix comparison of the deduced amino acid sequence from the largest open reading frame against that of the maize ACCase is presented in Figure 5. pK111 showed 82.33% homology with the maize cDNA at the nucleotide level and 88.17% similarity/78.44% identity at the amino acid level.

25 In addition the deduced amino acid data of the wheat cDNA showed large homologous regions with the known sequences of rat (62%) and yeast (62%) ACCase.

### 2.2 Isolation of a partial rape ACCase cDNA encoding the transcarboxylase domain.

30 Although ACCase has been purified from rape embryo, the amounts obtained were not amenable to protein sequencing. To study ACCase at the sequence level we needed to isolate its cDNA. A rape embryo derived poly dT primed  $\lambda$ ZapII library was screened with the partial wheat ACCase cDNA previously isolated. A hybridising cDNA of 2.5

kb was taken through three rounds of screening and plasmid rescued (pRS1). The clone was fully sequenced in both directions by a combination of nested deletions and dye primer sequencing. The cDNA sequence has been submitted to EMBL (Accession no. X77382). The predicted amino acid sequence from the largest open reading frame is shown in Figure 6. Dot matrix analysis of the cDNA with previously described ACCase sequences showed it to be a partial clone of ACCase corresponding to the transcarboxylase domain (Figure 7). The predicted amino sequence of the rape clone showed sequence identity/similarity levels of approximately 44/61% with the yeast (Al-Feel et al, 1992), rat (Lopez-Casillas et al, 1988) algae (Roessler and Ohlrogge, 1993) and the wheat ACCase cDNA pK111. Since the mRNA contains a polyA tail and was obtained from the poly A fraction it is probable that the ACCase cDNA isolated was nuclear encoded.

### 2.3. Isolation of the Arabidopsis ACCase genomic clone and further rape cDNA cloning

The average insert size of our rape poly dT primed cDNA library, described above, was approximately 2-2.5kb. Therefore it was unlikely that the library would contain much more 5' cDNA. To obtain more 5' sequence a random primed library from rape embryo mRNA was constructed. Having made a suitable library there were two strategies available for cloning more 5' cDNA i) screen using the 5' region of our cDNA, or; ii) screen using 5' probes from a genomic clone. We chose the second option. The strategy was to clone the ACCase genomic gene, identify the open reading frames by sequence comparison and generate specific probes by the use of PCR. Since Arabidopsis is related to rape and has a smaller genome we chose to obtain the genomic clone from Arabidopsis. Previous data from this laboratory had shown that Arabidopsis DNA



sequences are highly homologous to those of rape (data not shown). Screening a  $\lambda$  FixII Arabidopsis genomic library with a 1.2 kb XhoI/PstI fragment of the rape ACCase cDNA pRS1 yielded two independent genomic clones which  
5 hybridised strongly to the pRS1 ACCase probe. These were denoted  $\lambda$ AYE4 and  $\lambda$ AYE8.  $\lambda$ AYE8 was subcloned to produce two plasmids : pKLU81, a 5.3 kb subclone in the EcoRI site of pGEM 3ZF+ ; and pKLS2, which was excised from the  $\lambda$  clone by a partial SalI digest and subcloned into pSK+.

10 The pKLU81 subclone, considered to be a partial length genomic clone, was partially sequenced from the 5' and 3' ends. Therefore two sets of data are presented for the 5' and 3' sequences from the same clone. The nucleotide sequences, with the derived amino acid  
15 sequences from the 3 reading frames are shown in Figures 8 and 9. A data base search (Swissprot) using the derived amino acid sequence from the 5' 0.56kb DNA sequence showed 40% identity with chicken and rat ACCase (Figure 10).

The genomic clone (pKLS2) was extensively subcloned  
20 through a combination of EcoRI/SalI/XbaI/HindIII digests, and partially sequenced by both Dye primer and Dye terminator chemistry. We found that intron-exon boundaries could not be allocated without cDNA data. We therefore opted to sequence only enough of the genomic  
25 clone to allow generation of open reading frame probes for cDNA screening. The full sequence data obtained is shown schematically in figure 11A (hatched areas A,Aii,B,C,D,E and F) and has been deposited with the EMBL data base (accession no's X77375-X77381).

30 To map the ACCase activity domain order, within the genomic clone, the open reading frame sequences from the different sequenced areas were compared with the first two domains of the full length yeast cDNA (Figure 11Bi and ii), and the rape transcarboxylase domain (figure 11Biii).

Homology was sufficient to allow us to assign the same order of domains to the Arabidopsis gene as that of yeast ACCase shown in figure 11A ie: {Biotin carboxylase-Biotin binding-Transcarboxylase}.

5           Sequence data from an open reading frame at the 5' end of the genomic clone (area Aii) showed a marked homology (49.5/64% identity/similarity at the derived amino acid level) with the 5' region of yeast ACCase (see Figure 11Bi). The 3' end of the cloned genomic fragment  
10           (19kb) was sequenced and shown to be homologous to the 3' end of the rape 2.5kb cDNA clone isolated from the poly d'T primed mRNA library (figure 11Biii). Since we had approximately 1.3kb 5' to area Aii we reasoned that it was likely that pKLS2 was the full length genomic clone. The  
15           pKLU 81 subclone was a partial length genomic clone corresponding to a portion of the sequence of pKLS2.

          Since the Arabidopsis genomic clone showed a high degree of homology to the rape cDNA isolated (86% identity in the exons of areas E and F) it was clear that the  
20           genomic clone could be used to isolate further rape cDNA's. We generated a specific probe via PCR of area C within the genomic clone and used it to screen the random primed library generated from rape embryo mRNA. Two cDNA clones (pRS8 and pRS6 containing 2.0kb and 1.1 kb cDNA  
25           respectively) were isolated and sequenced. The cDNA from each was shown to overlap. The full combined derived amino acid sequence (pRS8/6 2.38 kb cDNA size) is presented in figure 12Ai (EMBL accession no X77374). The sequence analysis of the clones showed significant  
30           homology with that of yeast (39/58 % identity/similarity), rat (38/59% identity/similarity) and algal (34/54% identity/similarity) ACCase. Within the cDNA sequence is the highly conserved biotin binding site [Val-Met-Lys-Met], shown in figure 12Ai as the underlined region.

Direct comparison with yeast biotin binding site is shown in figure 12Aii. Interestingly the sequence also showed homology at it's 5' end with the 3' portion of the yeast biotin carboxylase domain. This data demonstrated that the domain order in rape [Biotin carboxylase-Biotin binding-Transcarboxylase] is consistent with the domain assignment of Arabidopsis.

### 3. Southern blot analysis

Since it was not known how many genes for ACCase are present in rape and Arabidopsis, total DNA was analysed by Southern blotting. Both rape and Arabidopsis total DNA was digested with three separate restriction enzymes and blotted. The Arabidopsis genomic clone indicated that ACCase genes would most likely be relatively large. The size dictated that it was not possible, using partial cDNA's as probes, to gain an accurate estimate of the gene copy number by Southern blot. The blot was therefore hybridised to the full Arabidopsisg genomic clone 19kb) labelled by random primed labelling. The sum of the Arabidopsis bands that hybrised to the probe was approximately 20kb (figure 13). Since the genomic clone is approximately 19kb, and showed a similar pattern when digested with the same enzymes (results not shown), we deduced that there is only the one gene present in Arabidopsis. Although the rape profile is more complicated it can be seen that it consists of a relatively small gene family (see figure 13).

### 4. Northern Blot analysis

The expression of ACCase during rape embryonic development was examined by Northern blotting using the 2.5 kb rape cDNA clone as probe. The blotrs contained 5 ug rape poly A+ m RNA prepared from a set of staged embryos taken from Brassica napus Jet Neuf at 15, 22, 29, 35, 42 and 49 days post-anthesis. Embryos taken from the same

seed set were also analysed for oil content to monitor development. The oil content data is presented (expressed as fatty acid/mg seed) graphically in Figure 14A. The Northern blot was hybridised separately to three successive probes and stripped after each in preparation for the next probe. The three probes used were embryo derived cDNAs for enoyl reductase (1.15 kb),  $\beta$ keto reductase (1.185 kb) and ACCase (2.5 kb). All three cDNAs were highly expressed in seed with maximum expression being coincidental at 29 days post-anthesis (Figure 14A). However it appears that the initial onset of mRNA production occurs in the order enoyl reductase,  $\beta$ keto reductase and ACCase. The profile of all three genes expression during embryogenesis was reproducible in individually probed blots with peak expression occurring at 29 days. The sizes of the hybridising bands were 1.65, 1.7 and 7.5 kb respectively as determined by size markers run on the same agarose gel used for the blot. The level of the ACCase mRNA was relatively lower than that of enoyl reductase and  $\beta$ ketoreductase. This may be in part due to the successive stripping of the blot and degradation of the large 7.5 kb message during handling.

A Northern blot comparison of ACCase expression in 29 days post-anthesis embryo and young leaf, using the embryo derived 2.5 kb cDNA as a probe is shown in Figure 14B. The 7.5 kb band that hybridises was approximately five times more abundant in seed than in leaf, as might be expected for ACCase. The size of the full length mRNA (7.5 kb) was consistent with the known size of the full length mRNA for both maize and wheat ACCase.

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## CLAIMS

1. Partial cDNAs specifying acetyl Coenzyme A carboxylase (ACCase), isolated from seed of Brassica napus, having the nucleotide sequence set forth in Figures 6 or 12, and variations thereof permitted by the degeneracy of the genetic code.
2. A partial cDNA specifying ACCase, isolated from wheat germ, having the nucleotide sequence set forth in Figure 4, and variants thereof permitted by the degeneracy of the genetic code.
3. A full length genomic DNA specifying ACCase from Arabidopsis thaliana having the nucleotide sequence set forth in Figure 8, and variants thereof permitted by the degeneracy of the genetic code.
4. The DNA inserts within the following clones, which have been deposited in Escherichia coli, strain DH $\alpha$  hosts, with the National Collection of Industrial & Marine Bacteria, 23 St. Machar Road, Aberdeen, AB2 1RY, United Kingdom, on 25th March 1993, under the provisions of the Budapest Treaty on the Deposit of Microorganisms for Patent Purposes, details of which

are as follows:

- 10 Plasmid pK111, Accession No. NCIB 40553;  
Plasmid pKLU81, Accession No. NCIB 40554, and,  
Plasmid pRS1, Accession No. NCIB 40555

- 5 5. A gene construct for use in transforming plants for the purpose of regulating expression of ACCase, comprising a promoter active in plant cells, a structural region encoding mRNA in sense or antisense orientation to one or more domains of the ACCase gene and a 3'untranslated region, whereby transformation of plant cells results in a phenotype characterised by reduced fatty acid production or the production of fatty acids of altered composition.
6. A construct as claimed in claim 5 in which the promoter is a tissue-specific or developmentally regulated promoter.
7. A construct as claimed in claim 6 in which the promoter is the promoter of the napin gene of Brassica napus.
8. A genetically transformed plant, plant cells and plant parts, containing a gene construct as defined in any of claims 5 to 7.

- 5 9. A genetically transformed plant and cells and parts thereof, having reduced ability to synthesise fatty acids or having the ability to sythesise fatty acids of altered composition, characterised in that the genome of said plant comprises a gene construct as defined in any of claims 5 to 7.
10. A genetically transformed plant as claimed in claim 9, in which the plant is an oil-synthesising plant.
11. A plant as claimed in claim 10, in which the plant is of the genus Brassicaceae.
- 5 12. A plant which is capable of synthesising polyhydroxyalkanoate polymer, characterised in that the the said plant contains within its genome a construct as claimed in any of claims 5 to 7 which reduces the ability of the plant to synthesise fatty acids from acetyl Coenzyme A and in addition genes directing synthesis of polyhydroxyalkanoate from acetyl Coenzyme A.
13. A method for the control of expression of ACCase in a plant comprising stably incorporating into the genome of the said plant by transformation a gene construct as claimed in any of claims 5 to 7.



14. A monocotyledonous plant having increased tolerance of herbicides which inhibit the activity of the ACCase endogenous to monocotyledonous plants comprising a monocotyledonous plant which has stably incorporated within its genome a DNA specifying ACCase, said DNA having been isolated from a dicotyledonous plant possessing a natural tolerance of the said herbicides.

15. A plant as claimed in claim 14 in which the herbicide is selected from the group consisting of arylphenoxy- propionate and alkylketone herbicides.

1 / 2 5

figure 1

fig 1A

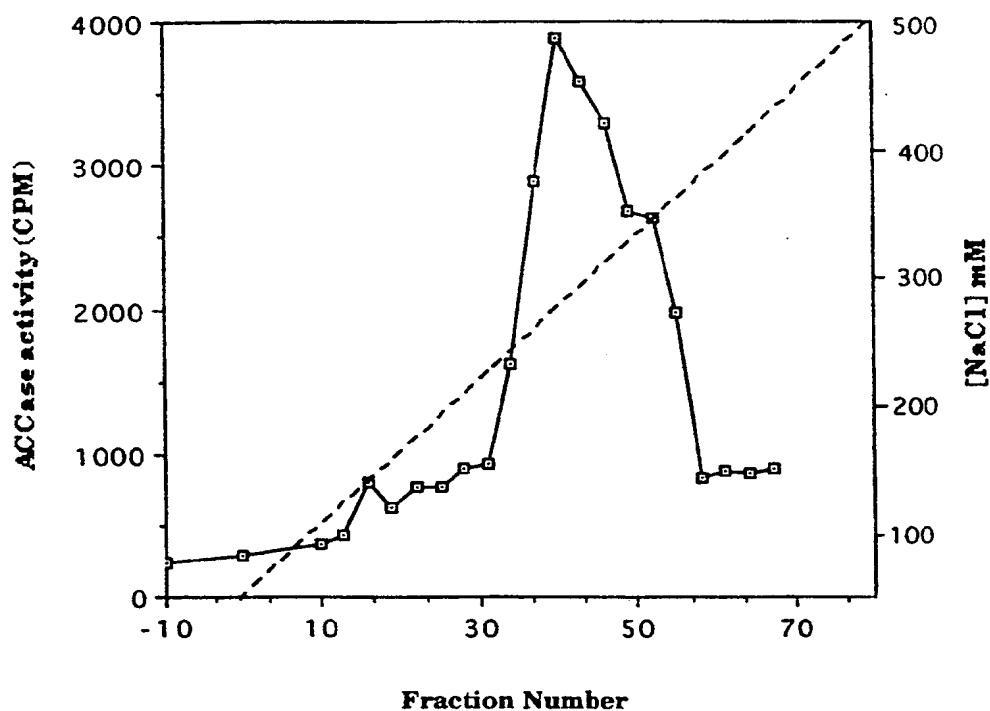
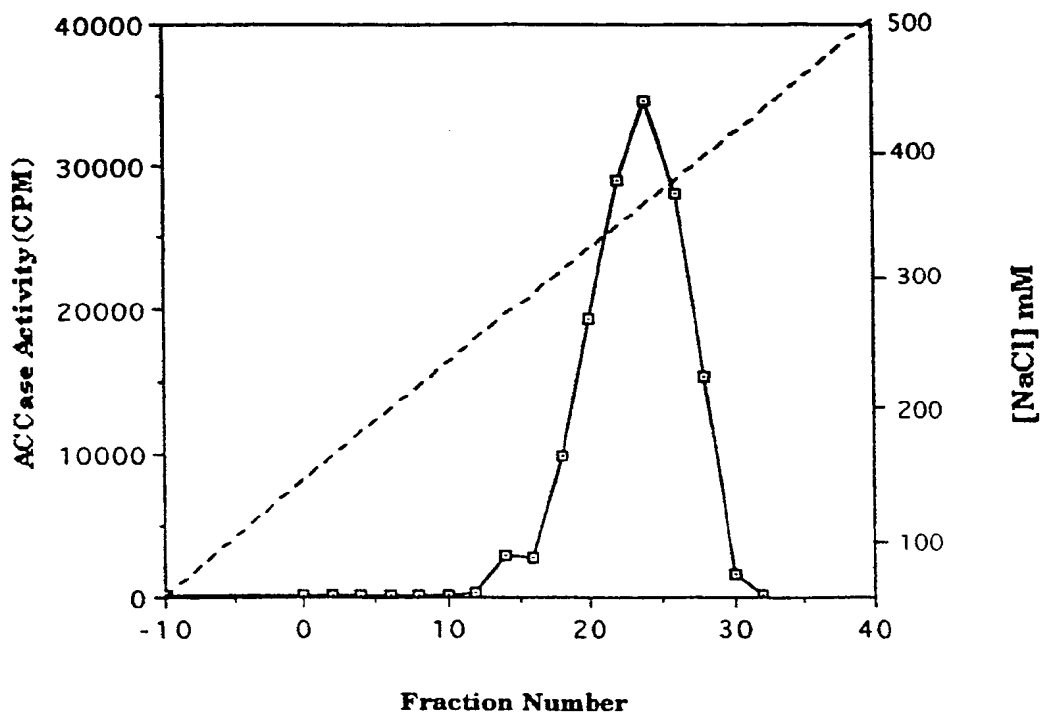
~~Q-sepharose chromatography (Wheat germ)~~

fig 1B

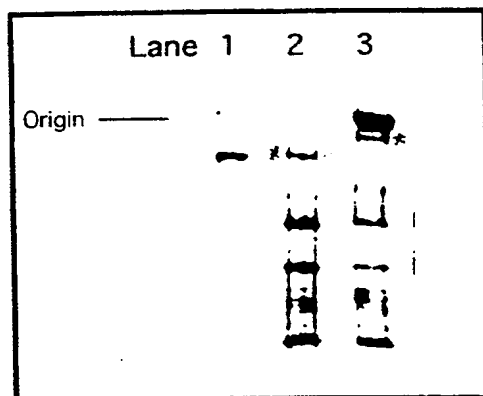
~~Blue-sepharose Chromatography (Wheat germ)~~

~~ACCase elution profiles for both Q-sepharose and Blue-sepharose column chromatography (see 1.2 for methods). The dotted line represents the NaCl gradient concentration and the activity of ACCase, represented by the boxes, was measured as in 1.1. Only the most active fractions were pooled for the next step in each case.~~

2 / 2 5

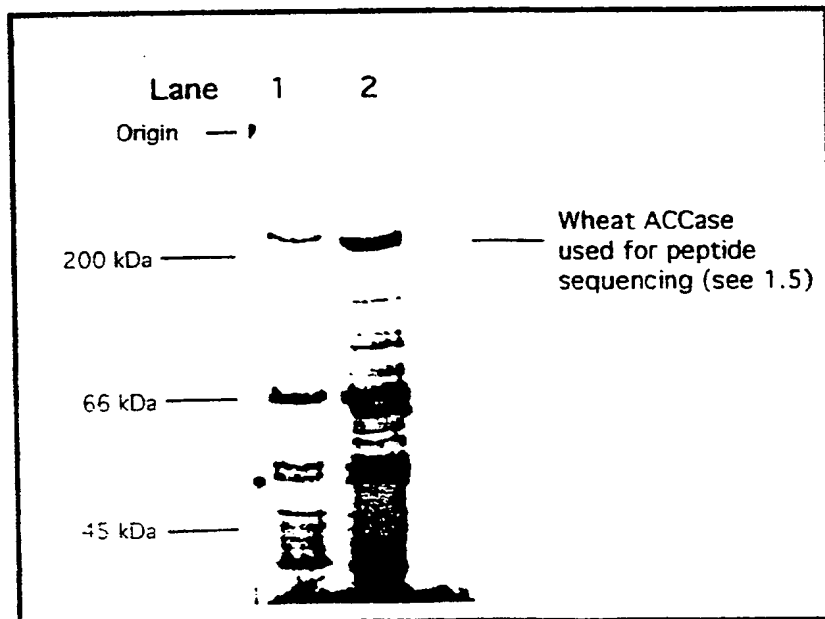
FIGURE 2

fig 2A

~~Wheat ACCase band shifting on addition of Streptavidin~~~~Lane 1 contains 500 ng Myosin (200 kDa)~~~~Lane 2 contains 10  $\mu$ l Post Blue-sepharose material without Streptavidin~~~~Lane 3 contains 10  $\mu$ l Post Blue-sepharose material with Streptavidin (see 1.4)~~

\* ACCase is indicated by the stars at its normal migration and that of the ACCase/streptavidin complex respectively

fig 2B

~~Wheat ACCase in Post Blue-sepharose material~~~~Lane 1 contains 1  $\mu$ l Post Blue-sepharose material (see 1.2 and 1.3 for methods)~~~~Lane 2 contains 10  $\mu$ l Post Blue-sepharose material~~

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F 2022 3.

## fig 3

~~Authentication of pK111 as Wheat ACCase partial cDNA~~~~For methods used see 1.3 and 1.5.~~

AA sequencing from Wheat ACCase    MATNGVE?LTVSDDLEG  
pK111 deduced AA sequence            81MATNGVVHLTVSDDLEG

AA sequencing from Wheat ACCase    LGGIPVGVIAVETQT?DQ  
pK111 deduced AA sequence            181LGGIPVGXIAVETQTMQ

AA sequencing from Wheat ACCase    NVLEPQGH  
pK111 deduced AA sequence            319NVXEXQLI

AA sequencing from Wheat ACCase    SIEARKKQLLPLYTQIAIRF  
pK111 deduced AA sequence            372SIEPRKKQLLPLYTQIAVRF

~~In order to authenticate the cDNA clone from wheat (pK111) Amino Acid sequence data was obtained. The direct comparison of AA sequence obtained to that deduced from the cDNA clone is shown. The numbers shown indicate the AA residue site of the sequence within that translated from the cDNA sequence.~~

4/25

## DNA Sequencer 1.2 ### Wednesday, February 3, 1993 10:14:05 am

FIGURE 4

WHEAT PK111 -&gt; 3-phase Translation

DNA sequence 1926 b.p. GAGAACATACAT ... \*TCCAGTTTTTT linear

```
1/1                               31/11
GAG AAC ATA CAT GGA AGT GCT GCT ATT GCC AGT GCC TAT TCT AGG GCC TAT GAG GAG ACA
E N I H G S A A I A S A Y S R A Y E E T
R T Y M E V L L L P V P I L G P M R R H
E H T W K C C Y C Q C L F * G L * G D I
61/21                               91/31
TTT ACG CTT ACA TTT GTG ACT TGA CGG ACT GTT GGA ATA GGA GCA TAT CTT GCT CGA CTT
F T L T F V T * R T V G I G A Y L A R L
L R L H L * L D G L L E * E H I L L D L
Y A Y I C D L T D C W N R S I S C S T W
121/41                               151/51
GGC ATA CGG TGC ATA CAG CGT ACT GAC CAG CCC ATT ATC CTA ACC GGG TTC TCT GCT TTG
G I R C I Q R T D Q P I I L T G F S A L
A Y G A Y S V L T S P L S * P G S L L *
E T V E T A Y * P A H Y P N R V L C F E
181/61                               211/71
AAC AAG CTT CTT GGC CGG GAA GTG TAC AGC TCC CAC ATG CAG TTG GGT GGC CCC AAA ATT
N K L L G R E V Y S S H M Q L G G P K I
T S F L A G K C T A P T C S W V A P K L
Q A S W P G S V Q L P H A V G W P Q N Y
241/81                               271/91
ATG GCG ACA AAC GGT GTT GTC CAT CTG ACA GTT TCA GAT GAC CTT GAA GGT GTG TCT AAT
M A T N G V V H L T V S D D L E G * V S N
W R Q T V L S I * Q F Q M T L K V C L I
G D K R C C P S D S F R * P * R C V * Y
301/101                               331/111
ATA TTG AGG TGG CTC AGC TAT GTT CCT GGC AAC ATT GGT GGA CCT CTT CCT ATT ACA AAA
I L R W L S Y V P A N I G G P L P I T K
Y * G G S A M F L P T L V D L F L L Q N
I E V A Q L C S C Q H W W T S S Y Y K I
361/121                               391/131
TCT TTG GAC CCA CCT GAC AGA CCC GTT GCA TAT ATC CCT GAG AAT ACA TGT GAT CCT CGT
S L D P P D R P V A Y I P E N T C D P R
L W T H L T D P L H I S L R I H V I L V
F G P T * Q T R C I Y P * E Y M * S S C
421/141                               451/151
GCA GCC ATC AGT GGC ATT GAT GAT AGC CAA GGG AAA TGG TTG GGG GGC ATG TTC GAC AAA
A A I S G I D D S Q G K W * L G G M F D K
Q P S V A L M I A K G N G W G A C S T K
S H Q W H * * P R E M V G G H V R Q R
481/161                               511/171
GAC AGT TTT GTG GAG ACA TTT GAA GGA TGG GCG AAG TCA GTA GTT ACT GGC AGA GCG AAA
D S F V E T F E G W A K S V V T G R A K
T V L W R H L K D G R S Q * L L A E R N
Q F C G D I * R M G E V S S Y W Q S E T
541/181                               571/191
CTC GGA GGG ATT CCG GTG GGT GT* ATA GCT GTG GAG ACA CAG ACT ATG ATG CAG CTC ATC
L G G I P V G X I A V E T Q T M M Q * L I
S E G F R W V X * L W R H R L * C S S S
R R D S G G C X S C G D T D Y D A A H P
601/211                               631/211
CCT GCT GAT CCA GGG CAG CTT GAT TCC CAT GAG CGG TCT GTT OCT CGT *CT GGG CAA GT*
P A D P G Q L D S H E R S V P R X G Q X
L L I Q G S L I F M S G L F L X L S K X
C * S R A A * F P * A V C S S X W A S X
661/221                               691/231
TGG TTT CCA *AT T*A *CT ACT AAG ACA GCT CAA GCA ATG CTG GAC TTC AAC CGT *AA GGA
W F P X X X T K T A Q A M L D F N R X G
G F X I X L L R Q L K Q C W T S T X K D
V S X X X Y * D S S S N A G L Q P X R I
721/241                               751/251
TTA CCT CT* TTC ATC CTT GC* AAC TGG AGA GGC TTC T*T GGT GGG CAA AGA GAT CTT TTT
L P X F I L X N W R G F X G G Q R D L F
Y L X S S L X T G E A S X V G K E I F L
T S X H P C X L E R L X W W A K R S F *
781/261                               811/271
AAA GGA ATC CTT CAG GCT GGG TCA ACA ATT GTT GAG AAC CTT AGG ACA TAC AAT CAG CCT
K G I L Q A G S T I V E N L R T Y N Q P
K E S F R L G Q Q L L R T L G H T I S L
R N P S G W V N N C * E P * D I Q S A C
```

5 / 2 5

Fig 4 (cont'd)

Whole AT pK111 - 2 phase Translation

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841/281 871/291  
GCC TTT GTA TAT ATC CCC AAG GCT GCA GAG CTA CGT GGA GGG GCT TGG GTC GTG ATT GAT  
A F V Y I P K A A E L R G G A W V V I D  
P L Y I S P R L Q S Y V E G L G S \* L I  
L C I Y P Q G C R A T W R G L G R D \* \*  
901/301 931/311  
AGC AAG ATA AAT CCA GAT CGA TTT GAG TTC TaT GCT GAG AGG ACT GCA AAG GGT AAT GTT  
S K I N P D R F E F Y A E R T A K G \* N V  
A R \* I Q I D L S S M L R G L Q R V M F  
Q D K S R S I \* V L C \* E D C K G \* C S  
961/321 991/331  
CT\* GAA CC\* CAA GGG TTG ATT GA\* ATC AA\* TTC AGG TCA GAG GAA CTC CAA GAG TGC ATG  
X E X Q G L I \* X I X F R S E E L Q E C M  
X N X K G \* L X S X S G Q R N S K S A W  
X T X R V D \* X Q X Q V R G T P R V H G  
1021/341 1051/351  
GGC AGG GTT GAC CCA GAA TTG ATA AAT CTG AAG GCA AAA CTC CTG GGA GCA AAG CAT GAC  
G R V D P E L I N L K A K L L G A K H D  
A G L T Q N \* I \* R Q N S W E Q S M T  
Q G \* P R I D K S E G K T P G S K A \* Q  
1081/361 1111/371  
AAT GGA AGT CTA TCT GAG TCA GAA TCC CTT CAG AAG AGC ATA GAA CCC CGG AAG AAA CAG  
N G S L S E S E S L Q K \* S I E P R K K Q  
M E V Y L S Q N P F R R A \* N P G R N S  
W K S I \* V R I P S E E H R T P E E T V  
1141/381 1171/391  
TTG TTG CCT TTG TAT ACT CAA ATT GCG GTG CGG TTT GCT GAA TTG CAT GAC ACT TCC CTT  
L L P L Y T Q I A V R F \* A E L H D T S L  
C C L C I L K L R C G L L N C M T L P L  
V A F V Y S N C G A V C \* I A \* H F P \*  
1201/401 1231/411  
AGA ATG GCT \*CT AAG GGT GTG ATT AAG AAG GTT GTA GAC TGG AAA GAT TCT AGG TCT TTC  
R M A X K G V I K K V V D W K D S R S F  
E W X L R V \* L R R L \* T G K I L G L S  
N G X \* G C D \* E G C R L E R F \* V F L  
1261/421 1291/431  
TTC TAC AAG AGA TTA CGG AGG AGG ATA TCC GAG GAC GTT CTT \*CA AAG GAA ATT AGA GGT  
F Y K R L R R R I S E D V L X K E I R G  
S T R D Y G G G Y P R T F X Q R K L E V  
L Q E I T E E D I R G R S X K G N \* R C  
1321/441 1351/451  
GTA AGT GGC AAG CAG TTC TCT CAC CAA TCA GCA ATC GAG CTG ATC CAG AAA TGG TAC TTG  
V S G K Q F S H Q S A I E L I Q K W Y L  
\* V A S S S L T N Q Q S S \* S R N G T W  
K W Q A V L S P I S N R A D P E M V L G  
1381/461 1411/471  
GCT TCT AAG GGA GCT GAA GCA GCA AGC ACT GAA TGG GAT GAT GAC GAT GCT TTT GTT GCC  
A S K G A E A A S T E W D D D D A F V A  
L L R E L K Q Q A L N G M M T M L L L P  
F \* G S \* S S K H \* M G \* \* R C F C C L  
1441/481 1471/491  
TGG AGG GAA AAC CCT GAA AAC TAC CAG GAG TAT ATC AAA GAA CTT AGG GCT CAA AGG GTA  
W R E N P E N Y Q E Y I K E L R A Q R V  
G G K T L K T T R S I S K N L G L K G Y  
E G K P \* K L P G V Y Q R T \* G S K G I  
1501/501 1531/511  
TCT CAG TTG CTC TCA GAT GTT GCA GAC TCC AGT CCA GAT CTA GAA GCC TTG CCA CAG GGT  
S Q L L S D V A D S S P D L E A L P Q G  
L S C S Q M L Q T P V Q I \* K P C H R V  
S V A L R C C R L Q S R S R S L A T G S  
1561/521 1591/531  
CTT TCT ATG CTA CTA GAG AAG ATG GAT CCC TCA AGG AGA GCA CAG TTT GTT GAG GAA GTC  
L S M L L E K M D P S R R A Q F V E E V  
F L C Y \* R R W I P Q G E H S L L R K S  
F Y A T R E D G S L K E S T V C \* G S Q  
1621/541 1651/551  
AAG AAA GTC CTT AAA TGA TCA GAT GAT ACC AAC GCA TCC AAT TCA GAA TGT GCA TGA TAT  
K K V L K \* S D D T N A S N S E C A \* Y  
R K S L N D Q M I P T H P I Q N V H D I  
E S P \* M I R \* Y Q R I Q F R M C M I S  
1681/561 1711/571  
CGG TTT CTC TTG AAG TAC ATA TAT AGA \*GG ATA CTA TTC GGC TGT AAC CGA CCA TAG CTG  
R F L L K Y I Y R X I L F G C N R P \* L  
G F S \* S T Y I X G Y Y S A V T D H S \*  
V S L E V H I \* X D T I R L \* P T I A D

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Fig 4 (contd)

~~Wheat pK111 - 3 phase Translation~~~~2/2/93 10:14:05 am Page 3~~

```

1741/581                               1771/591
ATC TGA GTC AAC CAT TAT TTT GTA AAA CTT TTT TGC GGT CTT CTC TGT TAT TCG AGG CAA
I  *  V  N  H  Y  F  V  K  L  F  C  G  L  L  C  Y  S  R  Q
  S  E  S  T  I  I  L  *  N  F  F  A  V  F  S  V  I  R  G  K
  L  S  Q  P  L  F  C  K  T  F  L  R  S  S  L  L  F  E  A  K
1801/601                               1831/611
AAC TTG TTT TCG GAC GGC TCC GAA TGG TTG ATG AGT GTA GTT GGA AAA AAA GCG GCC GGA
N  L  F  S  D  G  S  E  W  L  M  S  V  V  G  K  K  A  A  G
  T  C  F  R  T  A  P  N  G  *  *  V  *  L  E  K  K  R  P  E
  L  V  F  G  R  L  R  M  V  D  E  C  S  W  K  K  S  G  R  N
1861/621                               1891/631
ATT *CT GCA GCC CGG GGG ATC C*C TAG TTC TAG AGC GGC CGC ACC GGG TTG GAG *TC CAG
I  X  A  A  R  G  I  X  *  F  *  S  G  R  T  G  L  E  X  Q
  X  L  Q  P  G  G  S  X  S  S  R  A  A  A  P  G  W  X  S  S
  X  C  S  P  G  D  X  L  V  L  E  R  P  E  R  V  G  X  P  V
1921/641
TTT TTT
F  F
F
F

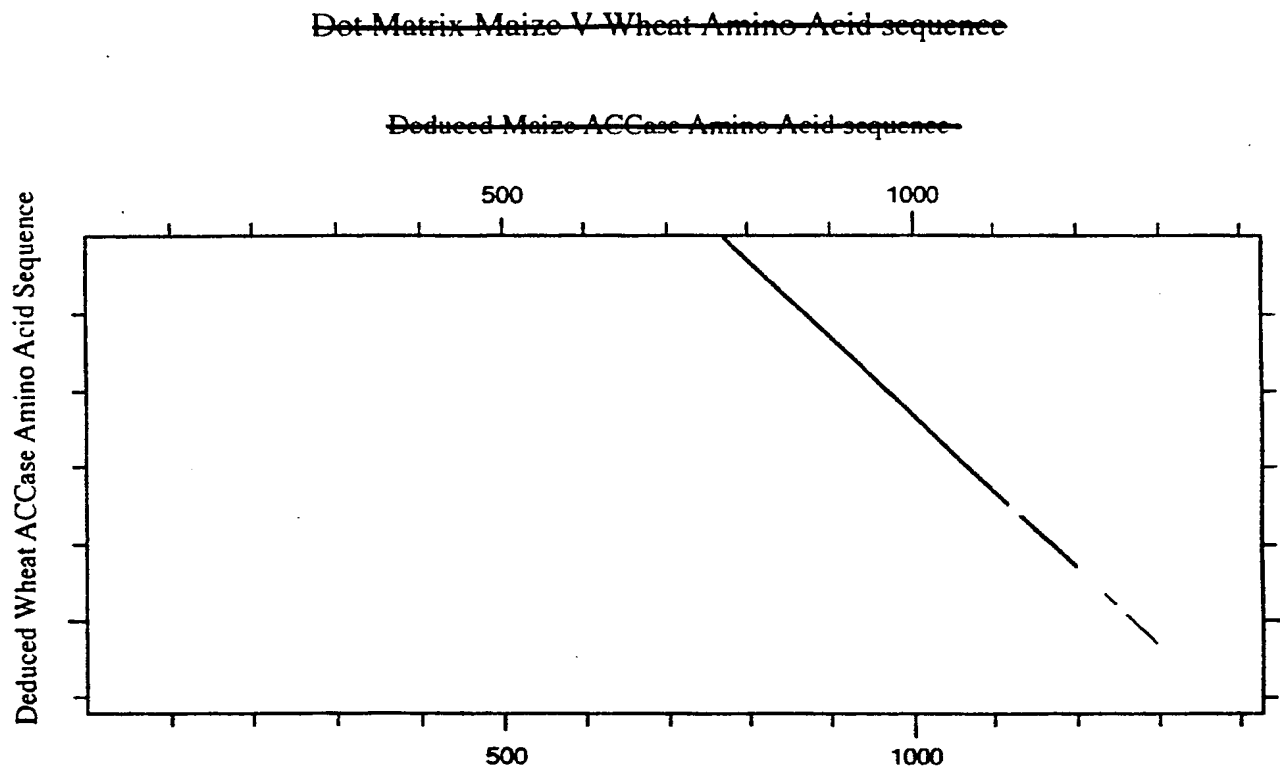
```

~~The sense strand of the Wheat cDNA sequence for pK111 with 3 Phase translation shown. The 1st phase is the translation from the largest open reading frame. The underlined sequences are those homologous to the Amino Acid data obtained for Wheat ACCase (see fig 3)~~

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## FIGURE 5

fig 5



~~Using DNA strider the two sequences were compared by dot matrix (stringency 15, window 23). The wheat sequence used was the 1st phase translation in respect to fig. 4. The Maize ACCase sequence was not derived.~~



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FIGURE 6A

1 ARGNSLIYH SITKGPLHG TQINDQYKPL GYLDRLAA  
 81 LKSLYPISE GTSMPVERS PGLNEFGVA WSLMSTPEF  
 161 PLYLAPNSG ARLGVAEEIK ACFKVGWSD VSPENGQYI  
 241 GVENLTGSG IAGAYSRAYN EFTLTFVSG RTVGIGAYLA  
 321 KINGTNGVH LTVSDLEGV SAILDWLSYI PAYVGGPLPV  
 401 DKNSFIETLE GMAITVTGR AKLGIPVGV VAVETQIVM  
 481 KSFHCLSLRT GEGFQVGREI FSKEYFRQVA TIVENLRITYR  
 561 NVLEPEGTIE IKFRTKEMLE CMGRDPKLI DLKARLQDPN  
 641 MRMTAKGVIK MCVEMIGSRS FFYKKLNRI AENSLVKNVR  
 721 TWKDNVSNEY LNLSELRQK LLNPTCRDMK FRRIYRCHK  
 RRSNTTYCYD FPLAFETALE QFGHYNNREL RNHARVLLSV  
 PMGRKLLIVA NDVTFKAGSF GPRDAFFLA VTELACPKL  
 YLSPEDHARI GSSVIAHEIK LPSGETRWVI DTIVGEDGI  
 PLGMRCIQRL DQPIILTFGS TLNKLGREV YSSHMQLGPG  
 LAPLDPDRIT VEYVENS CD PRAIAGVND NTGKMLGGIF  
 IIPADPGQLD SHERVPQAG QVMFPDSAGK TAQALMDFTR  
 QPVFVYIPKM GELRGAMV VDSQINS DYV EMYADETARG  
 QSEAYTNIEL LQQQIKAREK LLPVYIQIA TKFAELHDTIS  
 EASGDDL SYK SAMGLIQDMF SKSDIPKKE EAWTDDQVFF  
 DLPPIF 765

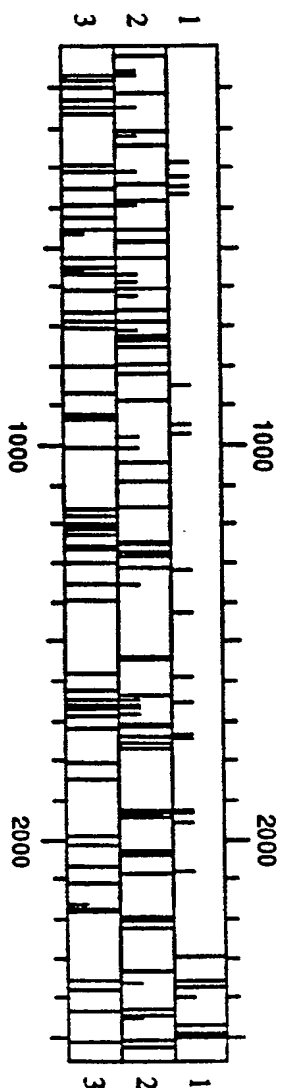


Figure 8b

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GCACGAGGGAGAAACAGTTTGATTTACCACTCAATTACCAAGAAGGGACCTTTGCATGGAA  
CCCAAATCAATGATCAATATAAGCCACTGGGATATCTTGACAGGCAACGTCTAGCCGCAAG  
GAGGAGTAACACTACATATTGCTATGACTTCCCGTTGGCATTGAGACAGCCTTGAGAGCAGT  
TTGGGCATTACAACAACCGGGAGTTAAGAAACCATGCAAGGGTACTCTTATCAGTGCTAAA  
GAGCTTGATTTCTCCAATTTTCAAGGTTACATCTCTTATGCCAGTTGAAAGATCACCGGGTC  
TCAATGAGTTTGAATGGTGGCCTGGAGCCTAGAGATGTGCGACTCCTGAGTTTCTTATGGG  
ACGGAAGCTTCTCATAGTCGCCAATGATGTACCTTCAAAGCTGGTTCTTTGGTCCTAGA  
GAGGACGCGTTTTTCTTGCCGTGACTGAACTCGCATGTCCAAGAAGCTTCCCTTGATTTA  
CTTGGCACCAAATTCTGGTGCCAGACTCGGAGTAGCTGAAGAAATCAAAGCCTGCTTTAAA  
GTTGGATGGTCGGATGAAGTTTCCCCGAAAATGGTTTTTCAGTATATATACCTAAGCCCTGA  
AGACCATGCAAGGATTGGATCATCTGTCAATTGCGCACGAAATAAAGCTCCCTAGTGGGGAA  
ACAAGGTGGGTGATTGATACAATCGTTGGTAAAGAAGATGGTATTGGTGTAGAGAATCTAA  
CCGGAAGTGGGCCAATAGCGGGCGCTTACTCGAGGGCATAACAACGAAACATTTACTTTGAC  
CTTTGTTAGTGGAAGAACGGTAGGAATTGGTGTCTTACCTTGCCCCCTTGGTATGCGGTGTA  
TACAGAGACTTGACCAGCCGATCATATTGACTGGCTTTTCTACGCTCAACAAGTTACTTGGG  
CGTGAGGTCTATAGCTCTCACATGCAACTTGGTGGCCCCGAAAATCATGGGCACAAATGGTGT  
TGTTTCATCTTACAGTCTCAGATGATCTCGAAGGTGTATCAGCGATTCTCGACTGGCTGAGCT  
ACATTCCTGCTTACGTTGGTGGTCTCTTCTGTTCTTGCCCCGTTAGACCCACCGGACAGAA  
CCGTGGAGTACGTTCCAGAGAACTCTTGCGACCCGCGAGCTGCTATAGCTGGGGTTAACGA  
CAATACCGGTAAATGGCTTGGCGGTATCTTTGATAAAAATAGCTTTATTGAGACTCTTGAAG  
GCTGGGCAAGAACGGTAGTGACTGGTAGAGCTAAACTAGGGGGAATACCTGTAGGAGTTG  
TTGCGGTTGAGACACAGACAGTAATGCAGATCATCCCAGCAGATCCAGGACAGCTCGACTC  
TCATGAAAGAGTGGTTCCACAGGCAGGGCAAGTCTGGTTTCTGATTCTGCGGGCAAGACA  
GCTCAAGCGCTCATGGATTTCACAAGGAAGAGCTTCCATTGTTTATCCTTGCGAACTGGAG  
AGGGTTTTTCAGGTGGGCAGAGAGATCTTTTCGAAGGAATACTTCAGGCAGGTTGCGACTAT  
TGTAGAAAATCTGAGAACGTATCGGCAGCCAGTGTGTTGTGTACATCCCTAAGATGGGAGAG  
TTGCGAGGTGGAGCGTGGGTTGTTGTTGATAGCCAAATAAATTCAGATTATGTTGAAATGT  
ATGCTGATGAAACTGCTAGGGGGAATGTGCTTGAGCCAGAAGGAACGATAGAGATAAAAT  
TTAGAACGAAAGAGATGTTAGAGTGCATGGGAAGGTTAGACCCGAAGCTAATCGATCTCAA  
AGCAAGACTGCAAGATCCCAACCAAAGTGAGGCTTATACAAATATCGAGCTCCTCCAGCAA  
CAGATTAAGCCCCGAGAGAAGCTTCTTACCAGTTTATATCCAAATCGCCACCAAATTTGC  
GGAACCTTCACGATACTTCCATGAGAATGACTGCCAAAGGAGTGATCAAAATGTGTGTGGAG  
TGGATCGGCTCGAGGTCCTTCTTCTATAAGAAGCTCAACCGGAGAATTGCTGAGAACTCTC  
TTGTGAAAAACGTAAGAGAAGCTTCAGGAGACGACTTATCGTATAAATCTGCAATGGGTTT  
AATTCAGGATTGGTTCTCCAAATCTGACATTCCAAGGGGAAAGAAGAAGCTTGGACAGAC  
GACCAAGTGTTCTTTACATGGAAGGACAACGTTAGTAACTACGAGTTGAATCTGAGCGAAT  
TGAGACCGCAGAAACTGTTGAACCCAACTTGCAAGAGATTGGAATTCGGTCGGATCTATCG  
GCGCTGCCACAAGGACTTGCCAATCTTCTAAACAAGGTGGAGCCTTCAAGAAGAGAAGAGC  
TTGTTGAAGCGCTACGAAAAGTGTTAGGTTGATGTACAAGAGGTCAAGCTTGTGACCCGAG  
AAAGATGGTCTTTGGTGTGCTTGTGTCCTACGGTGAAAGAAGCTAGTTGGAAATTAGAT  
GTGGTCTTTCTTTCTTAAATGTGTTGGCCCGAGCTGTAAATGTTGTTGTAGCGTATAAGTGA  
GAATTGCGTAATAATTTATTCAAC

FIGURE 7

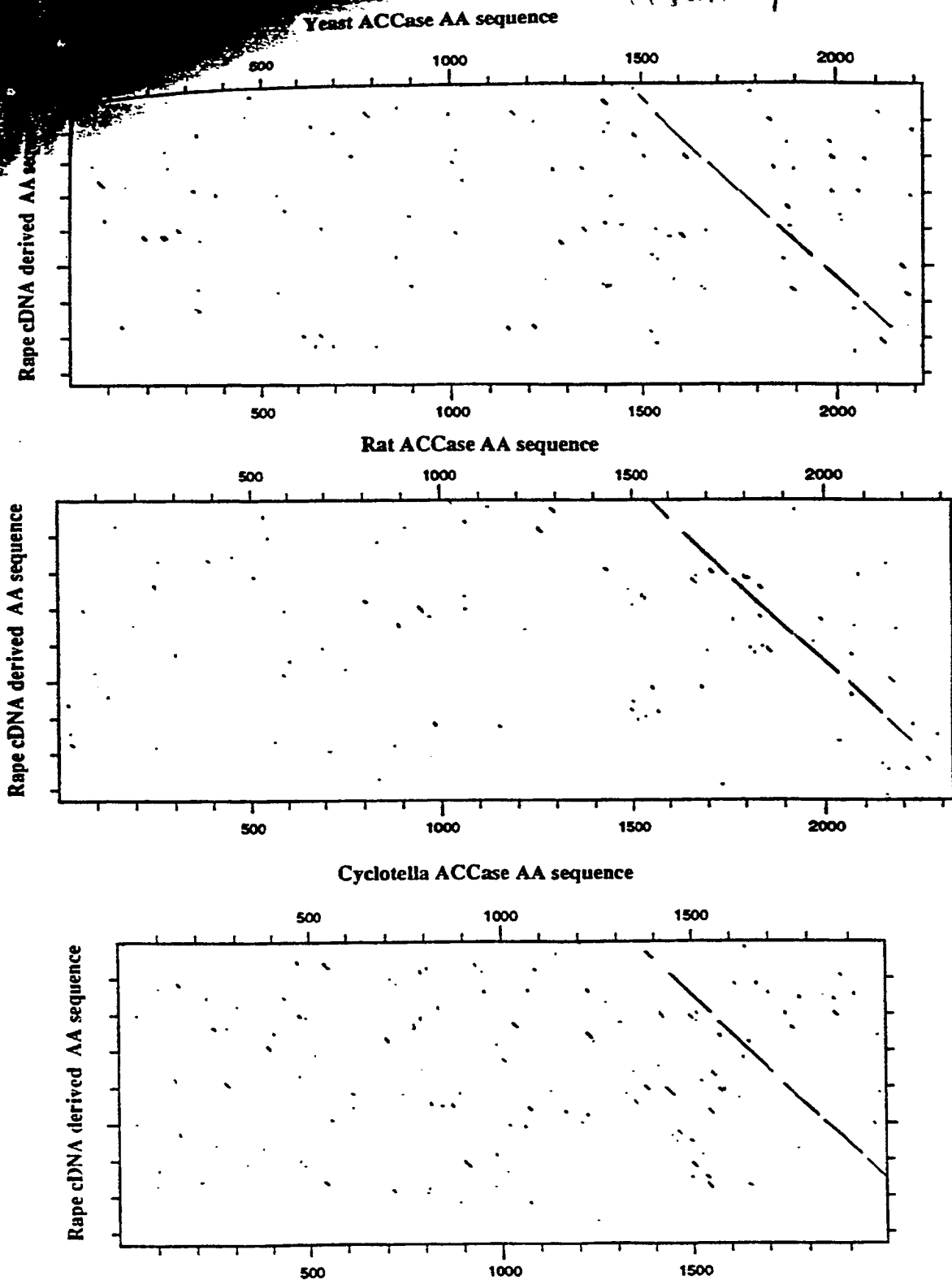


FIGURE 8

DNA sequence 565 b.p. GCGCTGCCAA ..... linear

~~The sense strand of the Arabidopsis sequence for pKLU81 with 3 Phase translation shown. The sequence shown is at the 5' end of the subclone. The 1st phase is the translation from the largest open reading frame.~~

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~~## DNA Sinder 1.2 ### Thursday, February 4, 1993 4:02:19 pm~~

FIGURE 9

~~pKLU81.15 - 3 phase Translation~~~~DNA sequence 255 b.p. tgcacccctggat tgcacccctggat linear~~

```

1/1                               31/11
tgc ccc ctg gat ggc atg tgg tgc ttg gag ggt tgt ggt tgc aaa cgt gac agg CCG TAC
C P L D G M W C L E G C G C K R D R P Y
A P W M A C G A W R V V V A N V T G R T
P P G W H V V L G G L W L Q T * Q A V H

61/21                               91/31
ATG CAC TGT CCA CGT AAG TTC CGC TTA CAA AAA ATT TGG TTG TAC AAG CAA TAC AGA GAG
M H C P R K F R L Q K I W L Y K Q Y R E
C T V H V S S A Y K K F G C T S N T E S
A L S T * V P L T K N L V V Q A I Q R V

121/41                               151/51
TAA GAG TAC ACA TCT CGA TGA CTT ACC TGC TGT GAT TTA ATA TTT CAG ATA TAC CGA GAA
* E Y T S R * L T C C D L I F Q I Y R E
K S T H L D D L P A V I * Y F R Y T E K
R V H I S M T Y L L * F N I S D I P R S

181/61                               211/71
GTT GAA ACT CCT GGA AGA AAC AGT TTA ATC TAC CAC TCA ATA ACC AAG AAG GGA CCT TTG
V E T P G R N S L I Y H S I T K K G P L
L K L L E E T V * S T T Q * P R R D L C
* N S W K K Q F N L P L N N Q E G T F A

241/81                               271/91
CAT GAA ACC CCA ATC AGT GAT CAA TAT AAG CCC CTG GGA TAT CTC GAC AGG CAA CGT TTA
H E T P I S D Q Y K P L G Y L D R Q R L
M K P Q S V I N I S P W D I S T G N V *
* N P N Q * S I * A P G I S R Q A T F S

301/101                               331/111
GCA GCA AGG AGG AGT AAC ACT ACT TAT TGC TAT GAC TTC CCG TTG GTT TGT TAC T
A A R R S N T T Y C Y D F P L V C Y
Q Q G G V T L L I A M T S R W F V T
S K E E * H Y L L L * L P V G L L

```

~~The sense strand of the Arabidopsis sequence for pKLU81 with 3 Phase translation shown. The sequence shown is at the 3' end of the subclone. The 1st phase is the translation from the largest open reading frame.~~

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## FIGURE 10.

~~Arabidopsis S<sup>1</sup> translated open reading frame FASTA against SWISSPROT Data base~~  
~~Scores shown are the two best fit~~

SCORES Init1: 87 Initn: 139 Opt: 125  
 38.4% identity in 73 aa overlap

		10	20	30	40	50
Arab		RPYFLQAAVPLLYPCTRIMYCLFLNFVEIRRFYGIEHGGYDSWRKTSVVA				
				11:::11::11:		::1::
Rat	PRLQVEHPCTEMVADVNLPAALQIAMGIPLFRIKDIRMMYGV-----SPWGDA-----					
	440	450	460	470	480	

	60	70	80	90	100	110
Arab	PFDFDKAQSIK-PKGHCVAVRVTSEXPDDGFKPTSGRVQVMZYLNNAKZKFIHZGTLWGN					
	1:11::: :: 1:11 ::1:111:11:11:11:11 11 ::					
Rat	PIDFENSAHVPCPRGHVIAARITSENPDGFKPSSGTQELNFRSNKNVWGYFSVAAAGG					
	490	500	510	520	530	540

	120	130	140	150	160	170
Arab	TCMNLQXLSEFKSKPNVWAYFSVKVIYIYXXSAIZVFHNXLIXRLLFYSCGAPXSNFHL					
Rat	LHEFADSQFGHCFSWGENREEAISNMVVALKELIRGDFRTTVEYLIKLETESFQLNRI					
	550	560	570	580	590	600

SCORES Init1: 87 Initn: 116 Opt: 117  
 46.5% identity in 43 aa overlap

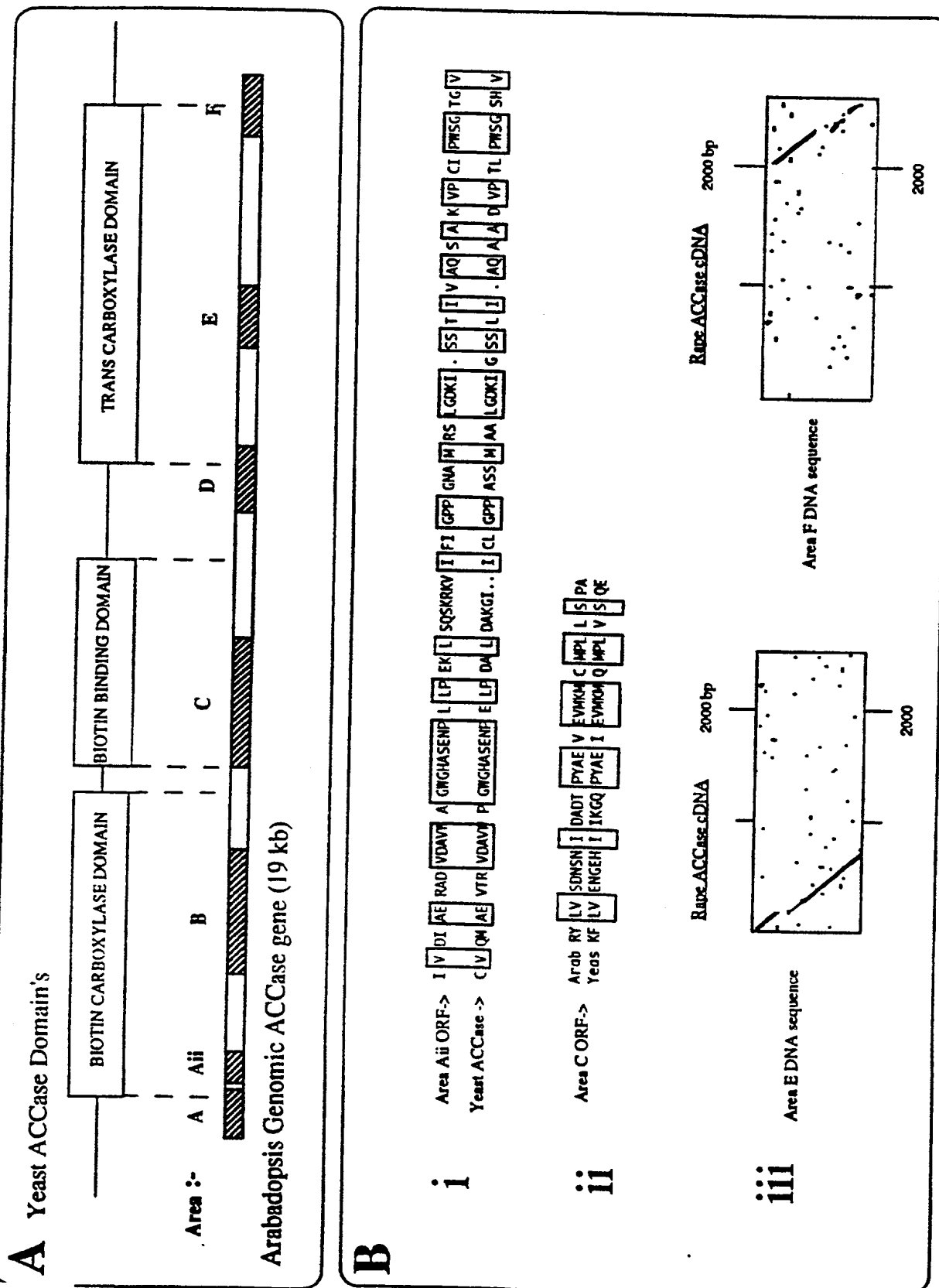
	30	40	50	60	70	80
Arab	LFNFVEIRRFYGIEHGGYDSWRKTSVVAFPDFDKAQSIK-PKGHCVAVRVTSEXPDDG					
				::11::: :: 1:11 ::1:111:11:11:11:11		
Chick	AAQLQIAMGIPLHRIKDIRVMYGVSPWGDGSIDFENSAHVPCPRGHVIAARITSENPDG					
	460	470	480	490	500	510

	90	100	110	120	130	140
Arab	FKPTSGRVQVMZYLNNAKZKFIHZGTLWGNTCMNLQXLSEFKSKPNVWAYFSVKVIYIYX					
	111:11 11 ::					
Chick	FKPSSGTQELNFRSNKNVWGYFSVAAAGGLHEFADSQFGHCFSWGENREEAISNMVVAL					
	520	530	540	550	560	570

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FIGURE 1A-15



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Figure 11c Region A.

TCGACTCGATCTGAAAATATCTAGTGTTCAACAACTTCAGATTCTTCGATCTACATATAAAT  
CTGTTACATTCTTTTTTTTATCAAAGAAATCACATTATTTTAGTAACTAATCCTAACTATAAA  
ATCTTTATTCAAGTATTTGATTATCCTTGATGAACTTTTAACAAACGGAATCAAATATAGGAA  
ACTAAATCGACCTATACAGAAAAATAATATTTAAATACAATACTTTTTTTTCTACTTAGCAC  
TTGGATGGCTTTATTGGCTTCATGATCTAGTGGAGCAAGATCAGTAGAGATTTGATATGGTT  
CAAGTTTGTTCTGGTCTAGTTTTTACGGGCATTTTTATGTACCTCGTGAACTTTCAAGTTATA  
AAATCCCGGTGCCTTGGAAGAAAAAGGTCTCAAAGACATAAGCATACAATAAAATTTGTTT  
TACAAAGTTTGGAACAAGTCAACGATGATTCGTTAATTTTCATTGCTAAAATGATTGGATCA  
TTCACAATTAACAAAAATGAGGAAAGAATGAGAGAAAGATGATAAGGTTGCCATACAATA  
TAAACCCATACCTAACTCTCAACTATATCTCAACCCCCAGTCATTTATAGTTACTATTAAGCC  
ATTAATATTATTTCTTTGTCAATGAGACCACTTTTATTCTCATTTTAAATAATCAAACAAAT  
GAAGAT



Figure 1 - Region H iii)

GAAGTACTATTATCTGAATTAACCGTGTTTTACTGTACAGAACACATGTATTAAGCTCAATTT  
CAGCAATGAAGTTTTGGTCTTTGGAGTTATTTGTCATTCATCTGAACATCTTTGTCTACAACC  
TGTGTGCAGATGGCTGAAGTAACACGCGTGGATGCAGTTTGGCCTGGTTGGGGTCATGCAT  
CTGAAAACCCCGAATTACCTGATGCCCTAGATGCAAAAGGAATCATATGTCCTTGGTCTCCA  
GCATCTTCAATGGCAGCACTGGGAGATAAGATTGGTTCTTCGTTGATTGCACAAGCTGCTGA  
TGTACCCACTCTGCCATGGAGTGGTTCCCATGTAAGTAAATTTACTCTTGTTAAGCTTGAGTA  
TTCTATAGTGTACCTAAATA

Figure C

GGAGGGTCCAATTACTGTGCTCCGCCAGAACTTTTCAAGAACTTGAACAAGCAGCTAGA  
AGGTTGGCTAAGAGTGTTAACTATGTTGGAGCTGCTACTGTTGAGTATCTCCACAGTATGGA  
CACTGGGGAGTACTACTTCTTAGAGCTTAACCCTCGCTTACAGGgtggttcatactgcagc  
ttttgcgttgaaatataatgaaggtccggacttgaaaattgaatgacttgtttaactgat  
gtttgagGTCAGGTTGAGCATCCTGTCACTGAGTGGATTGCCGAGATAAATCTTCCTTCTGCCCA  
AGATATACTGTGGGGATGGGAATTCCTCTCTGGCAAATCCCTGGTATAATCTACGTCCTTAT  
TTCTTACAGGcagcgggttcctctcttccatgcacagcaataatgtactgtctgtttct  
ctttaatttcgtagagataagacgggttctatggaatagaacatggaggagGTTATGATTCTT  
GGCGAAAAACATCTGTTGTAGCCTTCCCTTTTGATTTTGATAAAGCTCAATCTATAAGGCCA  
AAAGGTCATTGTGTGGCTGTACGTGTGACAAGTGAGGATCCTGATGACGGGTTCAAACCAA  
CCAGCGGTAGAGTTCAGGtaatgtatatctgtggaatgcaaagtgaagttcattcactg  
agaactctgtgggtaacactgtatgaactgcaacagGAGTTGAGTTTTAAGAGCAAGCC  
AAATGTGTGGGCGTACTTCTCTGTCAAGGtaattatatctatagagactctgctatataag  
tgtttcacaatgttttaattttacgactactttttacagtctggaggagGCATCCACGA  
GTTCTCG

Figure 1 - Region 2

ctatgtaagaacctctttctcagagATTTATTTGTCTTGAAAAGTTTCTATCTGGTGACGA  
AATGTTCTATCTGTCCAGAAAGCATCAGCGACCAGTGCTGCTGTGGTTTCAGATTACGTTGG  
TTATCTGGAGAAGGGGCAAATCCCTCCAAAGGtaatccaataccaggatctcttttgcctt  
tctagtgaatgttctgtagtaactttttctcttaacttgcagcatatctctgtaca  
ttctcaagtgtctcgaatattgaagGAAGTAAATATACGGTATTTCGCCTACTATCCAAATT  
TTACGTCTCTGCAATTTTCGTATTTTCTCTGCCATATTATTTTTGCGCTGAAGATATTGTTA  
CCAGgcttactaacaataaactgttctagagttagtagcaatgtagtccgggtggat  
cagGAACCTACAGGCTAAGAATGAACAAGTCAGAAGTGGTAGCAGAAATACACACTCTACGT  
GATGGAGGTCTGTTGATGCAAGcaagttttctgcctttttctataactacaagacaaggacat  
acatgtgtcgcgcagaaaaaacttctggagaatctcacttcttttctgttttcaactgtc  
attgcagttggatggcaaaagccatgtgatatatgcagagGAAGAAGCTGCAGGAACTCGTC  
TTCTCATTGATGGAAGAACTTGTGTTGCTACAGgtttctgctaattttttgtgtttacca  
tttacttcacgtttctcgaagtcatcttagcttttaagctgtctgtcaattttggctta  
ttcagAATGACCATGATCCATCAAAGTTAATGGCTGAGACACCGTGCAAGTTGATGAGGTAT  
TTGGTTTCTGACAAACAGCAATATTGACGCTGATACGCCCTTATGCCGAAGTTGAGGTCATGAA  
GATGTGCATGCCACTTCTTTCACCTGCTTCAGGAGTTATCCATCTTAAAATGTCTGAAGGAC  
AAGACATGCAGgtttcacttcattgctaacaacaaagctacagttctgtttaaattgattaa  
cccatccattattttttcacagGCTGGTGAACCTTATCGCCAATCTTGATCTTGATGATCCT  
TCTGCTGTAAGAAAGGCCGAACCCCTTCCATGGAAGTTTCCCAAGATTAGGGCTTCCAACTGC  
AATATCCGGTAGAGTTTCATCAGAGATGTGCCGCAACATTAAATGCTGCACGCATGATTCTTG  
CTGGCTATGAGCATAAAGTAGATGAGGtaaacactgtttgttttctatttgatccaactc  
tctctactagattatttgactatgagatagctcatagtcgcagGTTGTTCAAAGACTTACT  
TAATTGCCCTTGATAGCCCTGAACTCCCATTTCTTGCAGTGGCAACAGTGCTTTGCAGTTCTG  
GCGACACGACTACCTAAAAATCTCAGgaacatggtaaacacctgtgtagtattcataatccg  
gttcttatatattgatattttgtttgagttcaagacttttaatacatctaaataaaactct  
ttatcagctagaalcaagtatagGGAATTTGAGAGTATTTCCAGAAACTCTTTGACCACCG  
ATTTCCCTGCCAAACTTTTAAAAGGCAGTC

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Figure 11c Kyjin D.

CGAGTCAATTACTTGAACAGACCAAATAAGTGAAGCTTCGTTCAAACATTGCTAGAAGCC  
TTTCAGAGTTAGAAATGTTTACAGAGGACGGAGAAAAATATGGATACTCCCAAGAGGAAAA  
GTGCCATTAATGAAAGAATAGAAGATCTTGTAAGCGCATCTTTAGCTGTTGAAGACGCTCT  
CGTGGGACTATTTGACCATAGCGATCACACACTTCAAAGACGGGTTGTTGAGACTTATATT  
CGCAGATTATACCAAGgttcgagttcattctccgcacccttattgttcaaaattcttttg  
tactgcaattgattacagAAAATTTTGACTTCATTTTAACCCGACTCTTGTCATCAGCCCT  
ACGTCGTTAAAGATAGCGTGAGgatgcagtcgcgcggatgcagtggcaccttctggtct  
tcttgattcctgggatttctagagGAGCATATGGAAAGAAAAAACATTGGTTTAGACGAT  
CACGACACATCTGAAAAAGGATTGGTTGAGAAGCGTAGTAAGAGAAAAATGGGGGGCTATG  
GTTATAATCAAATCTTTGGAGTTTCTTCCACGTATAATACGTGCAGCATTGAGAGAAACAT  
AGCACAACGACTATGAAACTGCCGGAGCTCCTTTATCTGGCAATATGATGCACATTGCTAT  
TGTCGGGCATCAACAACCAGATGAGTCTGCTTCAGGACAGGTACTTGACACAGTAT

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Figure c Region E.

ACCGAGAAGTGAACCTGAAGAAACAGTTTAATCTACCACTCAATAACCCAAGAAGGGACCT  
TTGCATGAAACCCCAATCAGTGATCAATATAAGCCCCTGGGATATCTCGACAGGCAACGTT  
TAGCAGCAAGGAGGAGTAACACTACTTATTGCTATGACTTCCCGTTGgtttgttactgaat  
tcataagattcacacatacgttactcttttggctatttccaaccccccttatgttatttc  
tttccttttcagGCATTTGGGACAGCCTTGGAAGCTGTTGTGGGCATCACAACACCCAGGAG  
TTAAGAAACCATATAAGGATACTCTGATCAATGTTAAAGAGCTTGTATTCTCAAAACCAGA  
AGGTTCTTCGGGTACATCTCTAGATCTGGTTGAAAGACCACCCGGTCTCAACGACTTTGGA  
ATGGTTGCCTGGTGCCTAGATATGTCGACCCCAGAGTTTCCTATGGGGCGGAAACTTCTCG  
TGATTGCGAATGATGTCACCTTCAAAGCTGGTTCTTTTGGTCCTAGAGAGGACGCGTTTTT  
CCTTGCTGTTACTGAACTCGCTTGTGCCAAGAAGCTTCCCTTGATTACTTGGCAGCAAAT  
TCTGGTGCCCGACTTGGGGTTGCTGAAGAAGTCAAAGCCTGCTTCAAAGTTGGATGGTCGG  
ATGAAATTTCCCTGAGAATGGTTTTTTCAGTATATATACCTAAGCCCTGAAGACCACGAAA  
GGATTGGTCATCTGTCAATTTGCCCATGAAGGTAAAGCTCCCTAGTGGGGGAAACTAGGGTG  
GGGTGAATTGATACGGTCGTTGGGCAAAGAAGGATGG

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Figure c Key: F

gcaagctcgaaattaacccctactaaagGGAACAAAAGCTGGAGCTCTCTTGTA AAAA ACG  
TAAGAGAAGCATCTGGAGACAACCTTAGCATATAAAATCTTCAATGCGTCTGATTCAGGATTG  
GTTCTGCAACTCTGATATTGCAAAGGGGAAAGAAGAAGCTTGGACAGACGACCAAGTGTTT  
TTTACATGGAAGGACAATGTTAGTAACTACGAGTTGAAGCTGAGCGAGTTGAGAGCGCAGA  
AACTACTGAACCAACTTGCAGAGATTGGGAATTCCTCAGATTTGCAAGCTCTGCCACAAGG  
ACTTGCTAATCTTCTAAACAGggtataaaacaaaaccccccaaaaaacaaggttttggtc  
cccaagtaatcctaacctgtatgccggttttaagccctaagtaaatatttgtatgcaG  
GTGGACCGTCGAAAAAGAGAAGAGCTGGTGGCTGCTATTCGAAAGGTCTTGGGTTGACTGA  
TATCGAAGACTTTAGCTTCTAATCCAAGAAAGATGGACATTTAAAGTTTGCTTGTGTCCAT  
TTGGACCATCTTCCTTATATTTGTTGGTCACAGTTGTAAATGTTGTTGTAGCTTTGTCATT  
TCCGTATAAACAAATTACGCAATAATTCATTCAACATGTCACTCTTGCTTCATATTTATAC  
ACTGAACCAAGACAATATAATAGTCTAAATATAAACTGATCGGTCGACGCCCTATAGTGA  
GTCGTATTAAGCCGGCCGCGAGCTCTAGAGTC

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FIGURE 12.

A i

1	AGRLAKSVN	YVGAATVEYL	YSMDTGEYFF	LELNRLQVE	HPVTETIAEI	NLPAAQVAVG	MGIPLWQIPE	IRRFYGIENG	80
81	GGYDSWRKTS	VLSPDFEDK	AESIRPKGHC	VAVRTSEDP	DDGFKPTSGK	VOELSFKSKP	NVWAYFSYKS	GGGIHEFSDS	
161	QGHVFAFGE	SRALAIANW	LGLKNQNRG	KIRTWVDYTI	DLLHASDYRE	NQIHTCMLOS	RIAMVRAER	PPWYLSVYGG	
241	ALYKASATSA	AVVSDYGVY	EKGQIPPKHI	SLVHSQVSLN	IEGSKYTIQV	VRGGSGSYRL	RMHSEVVAE	IHTLRDGGLL	
321	MLQDGKSHVI	YAEFEAAGR	LLIDGRTCLL	QNDHDPKLM	AETPCKLLRY	LVSDHSSIDA	DAPYAEVEVM	KNCPLLSPA	
401	SGVIHFHMKSE	QAMQAGELI	AKLDLDDPSA	VRKAEPFNGG	FPRLGLPTAI	SGKVHQRCAA	TLNAARMVLA	GYEHKVDEVV	
481	QDLLNCLDSP	ELPFLQWQEC	FAVLATRLPK	DLRMWLESKY	REFESISRNS	LTADFPKALL	KGILEAHLIS	COEKDORGAL	
561	RLIEPIMLSLA	KSYEGGRESH	ARVIVHSLFE	EYLSVEELFN	DHMLADVIER	MRQYKKOLL	KIVDIVLSHQ	GIDKKNKLV	
641	RLMEQLVYPN	PAAYRDKLIR	FSTLNHTNYS	ELALKASQLL	EQTKLSELP	SNIAKSLSEL	EMFTEDGENM	DTPKRKSAIN	
721	ERMEDLVSSAS	LAVEDALVGL	FDHSDHTLIQR	RVVETVIRRL	YQPYVVKESI	RKQVHRSGLI	ASWFELEEHI	FRKHNH	796

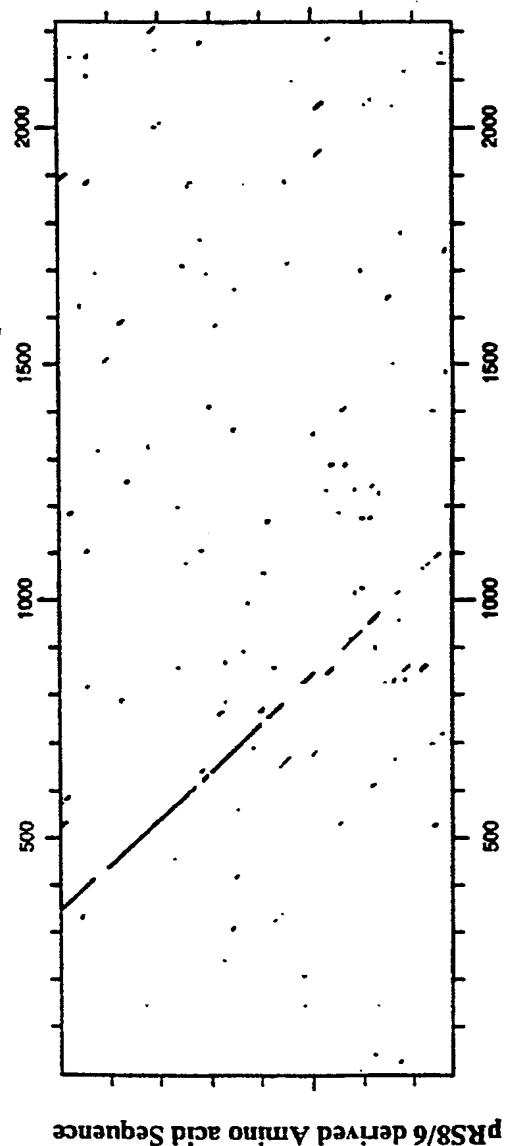
ii Yeast Biotin binding site AA 727

Rape derived AA 384

PYAE I	EVNKKM Q	MPL V	S
PYAE V	EVNKKM C	MPL L	S

B

Yeast ACCase Amino Acid Sequence



pRS8/6 derived Amino acid Sequence

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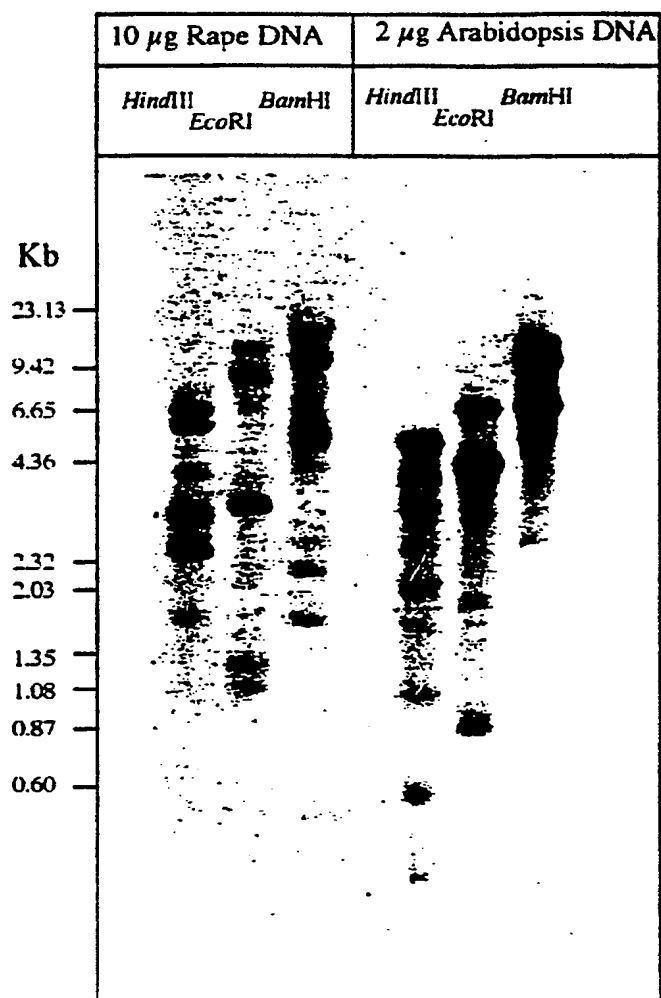
Figure 1c

TGGCTGGTAGAAGGTTGGCTAAGAGTGTTAACTATGTTGGAGCAGCTACTGTTGAATATCTC  
TACAGCATGGACACGGGGGAGTACTACTTCTTAGAGCTTAACCCTCGGTTACAGGTTGAGC  
ACCCTGTAACTGAATGGATTGCCGAGATAAATCTTCCTGCTGCGCAAGTTGCTGTTGGGATG  
GGAATTCCTCTCTGGCAAATCCCTGAGATAAGACGGTTCATGGTATAGAACATGGTGGAGG  
TTACGATTCTTGGAGGAAAACATCTGTGCTAGCCTCCCTTTTGATTTTGATAAAGCTGAATC  
TATAAGGCCAAAAGGTCATTGTGTGGCTGTACGCGTGACAAGTGAGGACCCTGATGACGGA  
TTCAAACCCACCAGCGGTAAAGTACAGGAGTTGAGTTTTAAAAGCAAGCCAAATGTGTGGG  
CTTACTTCTCTGTCAAGTCTGGTGGAGGCATCCACGAGTTCTCAGATTCCCAATTTGGCCATG  
TTTTTGCATTTGGGGAATCCAGAGCCTTGGCAATAGCAAATATGGTCCTTGGGCTTAAAAAA  
AATCAAAATCGTGGAAAAATTAGGACTAACGTTGACTACACGATTGACCTTTTACATGCTTC  
TGATTACCGGGAAAACCAAATTCACACTGGTTGGTTGGACAGTAGAATTGCTATGCGGGTC  
AGGGCAGAGAGGCCTCCATGGTACCTCTCTGTTGTCGGAGGGGCTCTCTATAAAGCATCAG  
CGACCAGTGCTGCTGTAGTCTCGGATTATGTTGGTTATCTAGAGAAGGGACAAATTCCTCC  
AAAGCATATATCTCTGTGCTTCTCAAGTGTCTCTGAACATTGAAGGAAGTAAATATACGA  
TTGATGTGGTCCGGGGTGGATCAGGAAGCTACAGGCTAAGAATGAACAACCTCAGAAGTTGT  
AGCAGAAATACACACTCTACGTGATGGAGGTCTGTTGATGCAGTTGGATGGTAAAGCCAT  
GTGATATATGCAGAGGAAGAAGCTGCAGGAACCCGCTCTCTTATTGACGGAAGAACCTTGT  
TACTTCAGAATGATCACGATCCTTCAAAGTTGATGGCTGAGACACCGTGCAAGCTGTGAG  
GTATTTGGTTTCAGATAATAGCAGTATTGATGCTGACATGCCCTACGCGGAAGTTGAGGTCA  
TGAAGATGTGCATGCCACTTCTTTACCTGCATCAGGAGTTATACATTTCAAAATGTCTGAA  
GGACAAGCCATGCAGGCTGGTGAACCTTATAGCCAAGCTTGATCTTGATGATCCTTCTGCTGT  
AAGAAAGGCCGAACCTTCCATGGAGGTTTCCCAAGATTAGGGCTTCCAACGGCAATTTCTG  
GTAAAGTTCATCAGAGATGTGCTGCAACTTTAAATGCTGCTCGCATGGTTCTTGCCGGCTAT  
GAGCATAAAGTAGATGAGGTTGTTCAAGACTTGCTTAACTGCCTTGATAGCCCTGAACCTCC  
ATTCTTCAGTGGCAAGAGTGCTTCGCAGTTCTGGCAACACGACTACCGAAAGATCTCAGAA  
TGATGTTAGAATCCAAGTATAGGGAATTTGAGAGTATATCCAGGAACCTCTCTCACC GCAGAT  
TTCCCTGCCAAACTTTTAAAAGGCATTCTTGAGGCTCATTATTATCTTGTGATGAGAAAGAT  
AGGGGTGCCCTTGAAAGGCTCATTGAACCATTGATGAGCCTTGCAAAGTCTTATGAAGGTG  
GTAGAGAAAGTCATGCCCGTGTTATTGTTCACTCTCTTTTGAAGAATACCTATCTGTAGAA  
GAATTATTCAATGATAACATGCTGGCTGATGTTATTGAACGCATGCGTCAGCAATACAAGAA  
AGATCTGTTGAAGATTGTTGATATTGTGCTCTCACACCAGGGCATTAAAGACAAAAACAAC  
TCGTTCTTCGGCTCATGGAGCAGCTTGTTTACCCTAATCCTGCTGCATACAGAGATAAACTTA  
TCCGATTCTCGACACTAAACCATACTAATTACTCTGAGTTGGCACTGAAGGCAAGCCAATTA  
CTCGAACAGACCAAATTAAGTGAACCTTCCAGCTTCAAACATTGCTAGAAGCCTGTCAGAGTT  
AGAAATGTTTACAGAGGATGGGGAAAATATGGATACTCCCAAGAGGAAGAGTGCCATTAA  
TGAAAGAATGGAAGATCTTGTGAGCGCATCCTTAGCTGTTGAAGATGCTCTCGTGGGACTA  
TTTGACCACAGCGATCACACACTTCAAAGACGAGTTGTTGAGACTTATATTGCGAGATTATA  
TCAGCCCTACGTCGTCAAAGAAAGCATCAGGATGCAATGGCACCGGTCTGGTCTTATTGCTT  
CTTGGGAGTTCTTAGAGGAGCATATTTCCGGAAACATTGGCTTA



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FIGURE 13.

~~ACCase Southern Blot Analysis of Rape and Arabidopsis genomic DNA~~

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FIGURE 14.

~~Fatty Acid Profile and Northern Blot Analysis of Rape Embryogenesis~~

Fig 14A

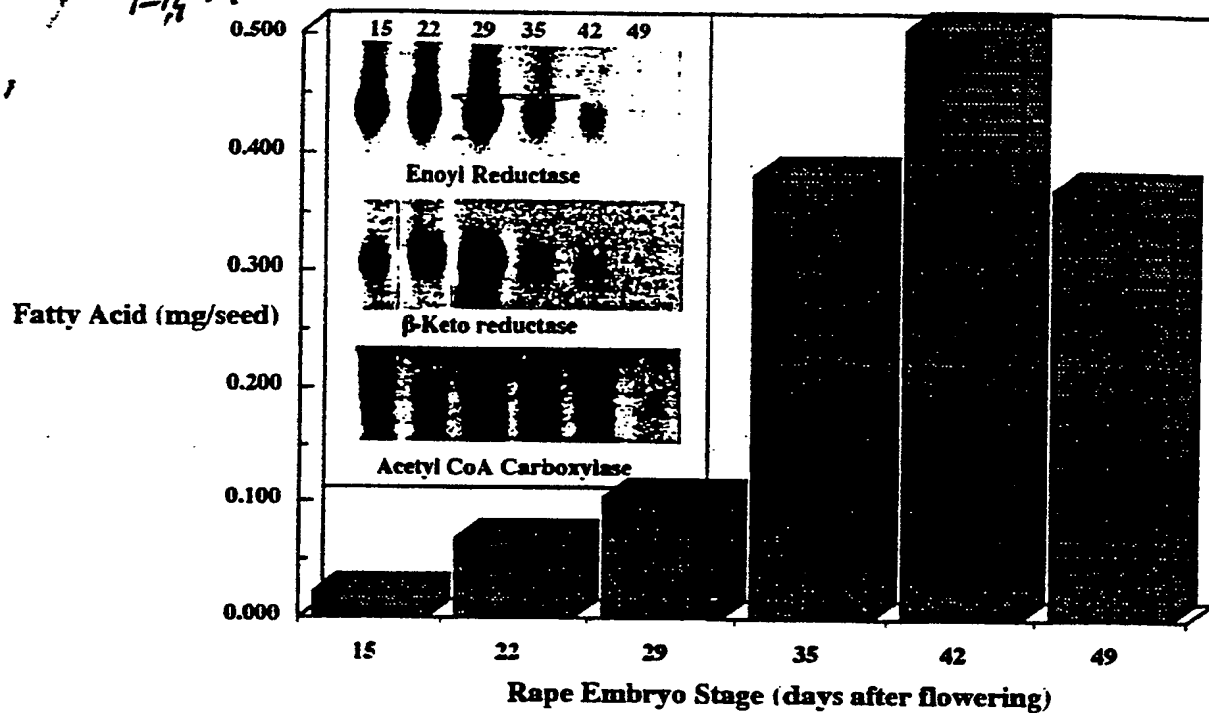
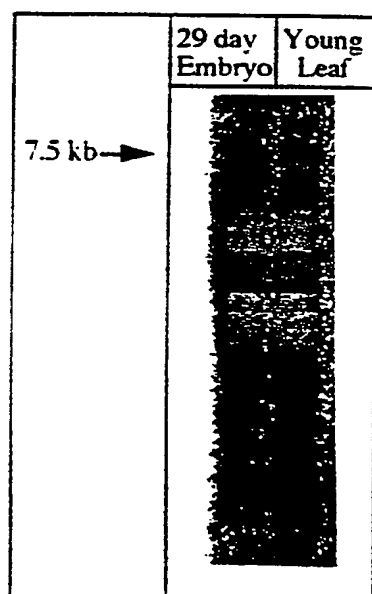


Fig 14B

~~B Northern Blot analysis of ACCase and Rape embryo V leaf expression~~

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 94/00846

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N15/52 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 December 1994

Date of mailing of the international search report

01.02.95

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## INTERNATIONAL SEARCH REPORT

Internal Application No

PCT/GB 94/00846

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International Application No

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Information on patent family members

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PCT/GB 94/00846

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